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Effects of excess dietary protein on ovarian function of beef cows

Taylor Clair Geppert
Iowa State University

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Taylor Clair Geppert

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Program of Study Committee:
Patrick Gunn, Major Professor
Daniel Loy
Howard Tyler
Patrick Phillips

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NOMENCLATURE

AA	amino acid
ADF	acid detergent fiber
AFC	antral follicle count
AI	artificial insemination
BCAA	branched-chain AA
BCS	body condition score
BW	body weight
CGM	corn gluten meal
CIDR	controlled intravaginal drug releasing device
CL	corpus luteum
CP	crude protein
CV	coefficient of variation
DDGS	dried distillers grains with solubles
DG	distillers grains
DM	dry matter
DMI	dry matter intake
FSH	follicle stimulating hormone
GH	growth hormone
GnRH	gonadotropin releasing hormone
GPCR	G protein-coupled receptor
IGF-1	insulin like growth factor-1
LH	lutenizing hormone

MCP	microbial crude protein
MP	metabolizable protein
MP125	125% of metabolizable protein requirement
MP150	150% of metabolizable protein requirement
mTOR	mammalian target of rapamycin
N	nitrogen
NDF	neutral detergent fiber
NE_g	net energy for gain
NE_m	net energy for maintenance
NRC	National Research Council
OT	oxytocin
PGC	primordial germ cells
$PGF_{2\alpha}$	prostaglandin $F_{2\alpha}$
PP	posterior pituitary
PUN	plasma urea nitrogen
RDP	rumen degradable protein
RUP	rumen undegradable protein
SBM	soybean meal
SEM	standard error of the mean
SD	standard deviation
SI	small intestine
TDN	total digestible nutrients
TMR	total mixed ration

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ABSTRACT

The effect of excess dietary protein supplementation on ovarian function of beef cows consuming low quality forage was investigated. In a pair of studies, non-pregnant, non-lactating beef cows were supplemented with excess dietary protein along with ad libitum access to chopped corn stalks. In the first study, the objective was to determine the effect of feeding excess metabolizable protein (MP) from feedstuffs differing in rumen degradability on ovarian parameters. Cows were supplemented with either a moderately undegradable protein (corn gluten meal; CGM) or low-undegradable protein (soybean meal; SBM) based supplement, formulated to supply 150% of NRC MP requirements for 58 days (d). Cows supplemented with CGM experienced greater dominant follicle growth post-dominance, larger follicles at spontaneous luteolysis, shorter duration of proestrus, and larger ovulatory follicles than SBM-treated cows. Peak estradiol production and corpus luteum volume did not differ due to treatment, but within the CGM treatment, cows tended to have lesser progesterone concentrations 7 d post-estrus compared to cows consuming the SBM supplement. Circulating plasma urea N (PUN) concentrations were elevated after supplementation in both treatment groups; yet no differences between treatments existed at ovulation. Total circulating plasma amino acid (AA) concentrations were not different between treatments after supplementation, but individual AA concentrations were shifted based on degradability of the protein supplement.

In order to determine if enhanced ovarian parameters observed in the first study was mediated by amount of undegradable protein, a second study was conducted offering

excess RUP at two inclusion levels. In the second study, cows were supplemented once daily with a moderately rumen undegradable protein (RUP) supplement, consisting primarily of corn gluten meal (62% RUP), at either 125% or 150% of NRC MP requirements for 60 d. Cows exposed to 150% MP had larger ovulatory follicles and greater antral follicle counts than cows in the 125% MP treatment. Estradiol and progesterone concentrations did not differ due to treatment; however, CL volume was greater in the 150% treatment group. Circulating urea N concentrations were elevated in both treatments at ovulation when compared to study initiation; however, PUN concentrations from cows consuming 150% MP exceeded concentrations of cows consuming 125% MP supplement after onset of the ovulatory follicle wave on interest. No differences in total circulating plasma AA concentrations existed between treatments before or after excess MP supplementation; yet, branched-chain AA were greater as a percent of total AA in cows offered 150% MP compared to 125% MP.

In summary, the data from these two studies indicate that degradability of feedstuffs, as well as inclusion, differentially affects ovarian functions of beef cows consuming a base diet of low quality forage. However, both studies demonstrate that excess undegradable protein fed to 150% of NRC MP requirements enhances ovarian function when compared to excess RDP or RUP supplementation at 125% MP. Therefore, we conclude that ovarian function of beef cows maintained on low quality forage can be enhanced by including excess protein from a moderately undegradable protein source at 150% of MP requirements and further research is warranted to translate effects to overall fertility.

CHAPTER 1.

INTRODUCTION

Between 1995 and 2014, beef cow inventory in the United States steadily declined, and in 2014 inventory reached record low numbers not seen since the early 1960s (USDA, 2015). More recently in the last 5 years, cattle inventory was further diminished in drought stricken areas of the country, such as the plains of Texas and Oklahoma, where producers were forced to reduce the size of their cow herd in order to maintain their operations. With the declining cow herd and shortage of economical feedstuffs in some parts of the country, cattle on feed inventory also decreased from 2009 to 2013 (LMIC, 2015a), as record high corn prices in relation to cattle prices took a lead role in determining the fate of some beef producers. In light of this, beef production remained steady (LMIC, 2015b) due to feeding cattle to heavier weights.

Moreover, between 1997 and 2012 Iowa alone lost 2 million acres of pasture land (US Census of Agriculture, 1997; 2012). This coupled with recent Midwestern droughts has forced producers to reevaluate their feed resources in order to meet nutrient demands of cattle without sacrificing profits; thus, new avenues of cattle feeding were explored. Specifically in the upper Midwest where corn is plentiful, producers have had the opportunity to decrease their feed costs during the winter with the use of corn stalks and coproducts stemming from the corn ethanol industry (Geppert and Gunn, 2014). Moreover, the reduction in pastureland acres has started to shift cow/calf production to a more intensified feeding system in parts of the Cornbelt, resulting in these stalk/coproduct diets being fed for longer periods of time, often into the breeding season.

With utilization of supplementing coproducts with low quality forages to beef cows, producers have seen improved reproductive performance compared to non-protein supplemented females (Funston et al., 2010). Therefore, coproducts have assisted in improving reproductive efficiency in the cow herd and historically have proved to be a cost effective addition to diets, allowing producers to begin growing their herds to meet the nation's demand for beef.

While distillers grains and other corn ethanol coproducts have shown to be an effective supplement in beef cow diets, some limitations exist and may become a concern for producers. When a coproduct is utilized as an energy source, more often than not the CP requirement of the cows is grossly exceeded due to the high protein content of the feedstuff. With excess CP consumption, biological and reproductive inefficiencies may occur due to alterations in metabolic parameters. While not fully investigated in beef cattle, excess protein supplementation has been associated with suppressed fertility in the dairy cattle industry (Butler et al., 1996). Moreover, as dairy cattle consume high concentrations of degradable protein to support milk production, the negative effects on reproduction may be due to the occurrence of a N energy imbalance. Nevertheless, contrary to dairy literature, excess CP stemming from supplementation of distillers grains has been shown to improve ovarian parameters of beef cows (Gunn et al., 2014b). However, while improved reproductive parameters were realized after distillers grain supplementation, this cannot be fully attributed to the dietary protein as the fat content of the supplement may have played a role in boosting reproductive functions (Staples et al., 1998).

With recent technological advancements of the ethanol industry striving to extract more value from the corn kernel, the emergence of fractionated coproducts with differing feeding values than traditional coproducts are impacting the beef industry (Berger and Singh, 2010). More specifically, these fractionated coproducts contain a different nutrient profile which has been particularly of concern when utilized in the feedlot industry; however, as these feeds are also used in cow/calf diets, the higher concentrations of protein and lesser levels of fat may differentially impact reproductive processes of beef cows. Therefore recent shifts in the way cows are fed in the Cornbelt, coupled with changing ethanol processes, foster the need to determine the effects of type and amount of excess dietary protein on ovarian function.

CHAPTER 2.

LITERATURE REVIEW

2.1 Introduction

The following chapter of this thesis contains a review of the literature relating to beef cattle reproductive function and the impact of protein supplementation in cow-calf diets. More specifically, the events of the estrous cycle are explained in detail including the specific endocrine mechanisms of which play critical roles in controlling the phases of the estrous cycle, follicular development and ovulatory follicle wave formation. Furthermore, a brief discussion on general nutrition and protein degradability is included, followed by the interactions between protein and reproductive functions in beef cows. Moreover, the specific interactions of excess protein supplementation and AA on ovarian function are discussed.

2.2 Endocrinology

2.2.1 Gonadotropin-releasing hormone

The principal regulator controlling reproduction, as well as the production and secretion of anterior pituitary hormones is gonadotropin-releasing hormone (GnRH), a decapeptide that is also historically known as lutenizing hormone-releasing hormone (Burgus et al., 1972). It was originally discovered in the porcine hypothalamus (Baba et al., 1971) in two main isoforms: GnRH–I and GnRH–II, which are thought to be important in reproduction. While GnRH-I is mainly responsible for regulating gonadotropin secretions, GnRH-II is thought to be associated with sexual behavior in

mammals (Chen and Fernald, 2008). Hypothalmic GnRH is secreted from neurons within the tonic center and surge center of the hypothalamus and travels through the hypophyseal portal system to the anterior pituitary (Moenter et al., 1992) where it acts on gonadotropes to synthesize and release the gonadotropins lutenizing hormone (LH) and follicle stimulating hormone (FSH; Shally et al., 1971; Wang et al., 2010). The tonic center of the hypothalamus (ventromedial nucleus and arcuate nucleus) is responsible for maintaining basal concentrations of GnRH by mediating pulses every 30 – 120 minutes throughout the luteal phase of the estrous cycle. The surge center of the hypothalamus (preoptic nucleus, anterior hypothalamic area and suprachiasmic nucleus) controls the preovulatory surge of GnRH necessary to initiate the LH surge that forces ovulation at the end of the follicular phase (Gorski, 1970).

When GnRH reaches the pituitary gland, GnRH stimulates production and release of gonadotropins by actively binding to G protein-coupled receptors (GPCR) on gonadotroph cell surfaces (Kakar et al., 1993). By binding to a GPCR, GnRH activates a series of pathways by signaling secondary messengers, increasing calcium stores, and activating mitogen activated protein kinases pathways (Weck et al., 1998). When these pathways are signaled, LH and FSH is synthesized and released from their storage location in the anterior pituitary and into systemic circulation to reach the ovaries. At the ovaries, the granulosa and theca cells bind FSH and LH, respectively. Through the 2-cell, 2-gonadotropin theory (Ryan and Petro, 1966), these cells use enzymes to produce progestins, androgens, and estradiol necessary to drive normal ovarian function.

2.2.2 Pituitary hormones

The heterodimeric glycoprotein hormones LH and FSH are synthesized and secreted from the anterior pituitary gonadotroph cells and are made up of two subunits: alpha and beta. The chemical structures of the two glycoproteins are similar with respect of their alpha subunits, but are differentiated based on unique beta subunits, which upon binding determine biological activity (Ryan et al., 1988). These glycoproteins are also coated in carbohydrates which are necessary for full biological responses of both subunits (Keutmann et al., 1983). When carbohydrate coverage increases on the subunits, biological activity is greatly enhanced as is their half life in circulation, while the opposite is true if they are deglycosylated. In the anterior pituitary, FSH is stored for shorter period of time than LH as it undergoes synthesis and secretion more frequently (Apfelbaum and Taleisnik, 1976). Lutenizing hormone has much more abundant storage in the anterior pituitary and is secreted at greater volumes than FSH, but at lesser frequencies (Muyan et al., 1994). The difference in storage between these two gonadotropins is most likely due to different responses to GnRH stimulus, biological functions and regulation of pathways (Farnworth, 1995), but both FSH and LH surges are important to facilitating normal estrous cycles.

2.2.2.1 Follicle stimulating hormone. Follicle stimulating hormone elicits slow pulse frequencies after a GnRH stimulus(Dalkin et al., 1989). One reason for this could be due to FSH half life of 149 minutes (Bogdanove and Gay, 1969), which delays feedback. However, surges of FSH can also occur without signal by GnRH, as evidenced by serum concentration increases during the luteal phase near day 1 and 8 when GnRH is

suppressed by progesterone (Sunderland et al., 1994). Therefore, non-GnRH inductions of FSH may be partially controlled through direct steroid hormone feedback on the anterior pituitary throughout the cycle (Nett et al., 2002) or theories of sustained GnRH presence, FSH releasing factors and sub-detectable GnRH (GnRH-II) also exist (Padmanabahn et al., 1997).

In cattle, FSH plays an important role in ovarian follicular wave emergence. In a study utilizing beef heifers, the FSH surge was determined to occur 1-2 days prior to wave emergence (Adams et al., 1992; Sunderland et al., 1994). The FSH surge is capable of initiating wave emergence due to presence of FSH receptors on granulosa cells of small (1-3 mm) and medium (4-6 mm) sized follicles (Adams et al., 2008). As the follicles continue to grow, FSH binding to FSH receptors on granulosa cells induces aromatase activity which elicits estrogen production within the follicle. When one follicle is selected for dominance, FSH also up-regulates LH receptors on the granulosa cells of large follicles (Ryle, 1972). Upon selection for dominance, circulating FSH concentrations are decreased due to production of estradiol and inhibin by the dominant follicle and granulosa cells, respectively (Adams et al., 2008). As the dominant follicle increases estradiol production, estradiol induces negative feedback on the anterior pituitary to decrease FSH which also halts further growth of subordinate follicles dependent on FSH to avoid atresia (Butler et al., 1983; Price and Webb, 1988). In addition, inhibin provides negative feedback to the anterior pituitary and suppresses FSH secretion by stopping the coding for FSH beta subunit (Carroll et al., 1989).

2.2.2.2 Lutenizing hormone. The secretion of LH more closely mimics the GnRH stimulus released from the anterior pituitary than FSH, potentially due to its shorter half life (30 min; Bogdanove and Gay, 1969). Aside from being controlled by GnRH, secretions of LH are also dependent on hormone concentrations concurrent with specific stages of the cycle. When estradiol is dominant during the follicular phase, it positively stimulates GnRH and induces the release of LH pulses with high frequency and low amplitude. By contrast, when progesterone dominates during the luteal phase, it suppresses GnRH and subsequently changes the pattern of LH surges to be released less frequently, but at greater amplitude than during the follicular phase (Rahe et al., 1980). However, when lysis of the corpus luteum (CL) occurs and progesterone suppression on estradiol is removed, positive feedback of estradiol on the surge center promotes LH release and allows LH to initiate high frequency, low amplitude pulses from the pituitary.

When LH is released from the anterior pituitary, it targets LH receptors on theca cells of the antral follicle. Upon binding to theca cells, LH signals progestins to be converted to androgens which are then transferred to granulosa cells for estradiol production during the follicular phase (Ryan and Petro, 1966). As the follicle matures and becomes selected for dominance, LH receptors also form on granulosa cells allowing the follicle to switch from FSH to LH dependency (Driancourt, 2001). Once follicles switch to LH dependency, LH assumes the role of regulating growth of ovulatory follicles (Ryle, 1972) as well as initiates final maturation of the oocyte (Driancourt, 2001). When a follicle has attained dominance, elevated estradiol production in follicular fluid provides positive signals to the surge center and drives GnRH surge which increases frequency of LH pulses, leading to the preovulatory LH surge and ovulation 25-35 h later (Chenault et

al., 1975). Once ovulation occurs, the developing CL begins producing progesterone which is assisted by LH for normal progesterone secretion between d 2 and 12 of the luteal phase (Peters et al., 1994).

2.2.3 Steroid hormones

2.2.3.1 Estradiol. The primary hormone required for visual display of estrus is estradiol, which is also the primary hormone secreted from the dominant or Graafian follicle. Estradiol is synthesized through enzyme activity within granulosa cells of the follicle. When estradiol synthesis begins, estradiol concentrations amplify in the follicular fluid and within systemic circulation, which eventually reach concentrations capable of initiation of greater LH pulses prior to ovulation (Rhodes et al., 1995). Estradiol concentrations peak following luteolysis of the CL and before the LH surge (Walters and Schalenberger, 1984). Throughout the follicular wave, estradiol concentrations fluctuate with the growing follicles but remain relatively low during luteal phase due to inhibitory actions of progesterone on LH and estradiol (Hansel et al., 1973).

2.2.3.2 Progesterone. Progesterone is the main hormone secreted during the luteal phase (Donaldson and Hansel, 1965) and is produced mainly from the large luteal cells of the CL which developed from granulosa and theca cells of the luteinized follicle (Smith et al., 1994). Progesterone increases rapidly during the first 3 days after ovulation and peaks near 4 ng/mL between d 10 and 14 in cattle (Adams et al., 2008). Progesterone is coordinated with neuroendocrine mechanisms in regulating pulse frequencies of LH and thus suppresses endogenous estradiol during the luteal phase (Short et al., 1979).

Progesterone is also the main luteotrophic substance important in maintaining the CL, preparing the uterus for implantation and maintaining pregnancy when fertilization is successful in the bovine.

2.3 The estrous cycle

2.3.1 Overview of the estrous cycle

The average length of the bovine estrous cycle is 21 days and can range from 17-24 days (Wishart, 1972; Salisbury et al., 1978) depending on age, genetics, and follicular wave pattern of the female. During the estrous cycle, two phases exist: 1) follicular phase, which consists of the proestrus and estrus periods and 2) luteal phase, which is made up of the metestrus and diestrus periods. These phases and periods of the cycle are characterized by specific gonadotropin and hormone interactions with the hypothalamic-pituitary-gonadal-axis. Estrus lasts for approximately 12 to 18 hours, with ovulation occurring 24 to 48 hours after onset of estrus, or 10 to 14 h after estrus ends (Hammond, 1927; Nalbandov and Casida, 1942). Thus, in each bovine estrous cycle, cows only have one opportunity to become pregnant and must be inseminated during the estrus period.

2.3.2 Follicular phase

The follicular phase is 4-6 days long, consisting of the proestrus (d 17-20) and estrus (d 21-1) phases of the estrous cycle, which is the time between lysis of the CL to ovulation (Sunderland et al., 1994). As the follicular phase begins with destruction of the CL, circulating progesterone characteristically decreases and removes its negative feedback on estradiol and LH pulse frequencies. When positive feedback of estradiol

reaches the surge center and the LH surge is initiated, the preovulatory follicle is able to undergo final growth and maturation (Adams et al., 2008). During this phase when the preovulatory follicle reaches maximum estradiol production, the female begins exhibiting behavioral estrus and is physically receptive to being mated. This is usually termed estrus or ‘day 0’ of the cycle. Aside from promoting follicle growth and maturation, the increasing estradiol concentrations from the dominant follicle propagates positive feedback to the surge center of the hypothalamus and induces formation of estradiol receptors on the anterior pituitary, allowing the preovulatory surge of LH and ovulation of a dominant ovulatory follicle to occur (Nett et al, 1987).

2.3.3 Luteal phase

The luteal phase encompasses a majority of the estrous cycle as it is 14-18 days in duration and is comprised of metestrus (d 2-4) and diestrus (d 5-17). This phase begins upon ovulation of the dominant follicle, approximately 30 h after the LH surge which is initiated by estrus (Nalbandov and Casida, 1942). The primary characteristic of the luteal phase is presence of a CL on the ovary providing production of the progesterone. During metestrus, progesterone concentrations drastically increase from < 1ng/mL to 3 ng/mL (Adams et al., 2008). This rise in progesterone is correlated with the developing CL, as the granulosa and theca interna cells of the ovulated follicle are undergoing lutenization and begin to form the large and small luteal cells of the CL, respectively (Hansel et al., 1973). Lutenization is a process of turning cells from the former fluid filled follicle into a vascular structure, through tissue remodeling and cellular proliferation, capable of secreting progesterone (Smith et al., 1994). During diestrus, the CL reaches peak

progesterone production of > 4 ng/mL near d 10 and maintains this concentration through late diestrus (d 16) until luteolysis (Adams et al., 2008). Once the CL is developed, proper function is maintained with the use of multiple growth factors and receptors supplying adequate blood flow, nutrients and hormones (Tamanini and Ambrogi, 2004). If pregnancy is established by presence of a conceptus, the antiluteolytic signal of the bovine, interferon tau, is produced which will block prostaglandin release from the uterine endometrium and prevent lysis of the CL (Bazer et al., 2009). However, if implantation is not established, progesterone will be down-regulated by luteolytic mechanisms cutting off blood supply and nutrients supporting the function of the CL, and the female will return to estrus.

2.3.4 Luteolysis

Luteolysis is the mechanism by which the CL of an actively cycling animal is caused to regress by luteolytic substances such as prostaglandins, primarily prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; McCracken et al., 1999). However, estrogens have also been shown to stimulate luteolytic activity in ewes (Warren and Hawk, 1971) and guinea pigs (Blatchley et al, 1971). It was first discovered that $PGF_{2\alpha}$ is produced by the endometrium in ruminants and other domestic species when Loeb (1923) performed hysterectomies on guinea pigs and the CL failed to regress. Progesterone signals $PGF_{2\alpha}$ to be released from the uterine epithelium in a pulsatile manner if no recognition of a pregnancy signal is received by uterine endometrium. The signal to lyse the CL is received in the posterior pituitary (PP) gland which begins release of oxytocin (OT; McCracken et al., 1999). When frequent pulses of OT are released from the PP, OT binds to receptors on the

uterine endometrium, and $PGF_{2\alpha}$ secretion from the uterus is initiated in a positive feedback mechanism (Flint et al., 1986). The $PGF_{2\alpha}$ that is produced by the endometrium travels through the bloodstream into the uterine vein, where it interacts in a countercurrent exchange with the ovarian artery (Ginther et al., 1981) to reach the CL and begin luteolysis via vasoconstriction and apoptosis (Nett et al 1976). Additional OT released from the CL also encourages release of supplemental $PGF_{2\alpha}$ from the uterine endometrium to aid in lysis of the CL (Silvia et al., 1991).

2.4 Ovarian follicular development in cattle

2.4.1 Ovarian reserve establishment

The ovary of all females houses the ovarian reserve which is comprised of a pool of primordial ovarian follicles. All follicles that reach the ovarian reserve originate from primordial germ cells (PGC) which are developed from the inner cell mass of the embryo. As the embryo develops, the PGCs migrate through the mesonephros to the gonadal ridge where they proliferate and develop in the ovary and establish the ovarian pool of follicles (Picton, 2001). The number of follicles in the pool is highly variable between females and is thought to be determined during fetal development, peaking at 2.7×10^6 at d 110 of gestation and falling quickly to 133,000 at birth due to apoptosis (Erickson, 1966). It should be noted that PGCs continue to decrease with age, as by the time the female reaches puberty there are less than 20% of the original PGCs remaining (Faddy et al., 1987). It is not known exactly why so many PGCs undergo apoptosis before puberty; however, possibilities include genetic errors or metabolic disturbances. As it is thought that the follicular reserve is determined prior to birth, there is no known

system that continually replenishes the pool. However, current research is looking at utilization of maternal nutrition to influence the size of the ovarian pool during fetal development (Cushman et al., 2014).

2.4.2 Follicle classification

There are three categories of follicles that develop from the PGCs and form follicular waves which include primary, secondary and tertiary or antral follicles. Primordial follicles are the smallest category of follicles within the ovary. Primordial follicles are the fundamental structure of the ovary that contains the germ cell surrounded by a single layer of squamous, pre-granulosa cells (Picton, 2001). Primordial follicles are not gonadotropin dependent as excess gonadotropin treatment does not seem to alter growth (Picton, 2001). When the primordial follicles begin meiosis upon signaling via kinase pathways (Tripathi et al., 2010), they are classified as primary follicles which are distinguished from primordial follicles by a larger oocyte and the squamous pregranulosa cells develop into a single layer of cubodial granulosa cells (Braw-Tal and Yossefi, 1997).

Follicles transition from primary to secondary follicles when 2-6 layers of granulosa cells are developed in late secondary stages. As the secondary follicle transitions to the preantral phase, stromal cells develop the theca interna cells and zona pelucida which surrounds the granulosa cell layers (Braw-Tal and Yossefi, 1997). When an antrum (or fluid filled cavity) develops within the layers of granulosa and theca cells, the follicle is transitioning into a tertiary or antral follicle. Antral follicles are dependent on and responsive to the gonadotropins LH and FSH, which are necessary to enter

follicular waves and complete ovulation. In addition as the follicle grows to secondary and tertiary (antral) sizes, the oocyte will remain in prophase-I until meiosis is reactivated by peak estradiol and the LH surge prior to ovulation (Ayalon et al., 1972).

2.4.3 Folliculogenesis

Folliculogenesis, or ovarian follicular growth, is the process of a synchronized group of primordial follicles leaving the resting pool and developing into secondary and dominant follicles capable of attaining dominance and ovulation (Savio et al., 1988). The follicles that make up a particular follicular wave develop over a number of weeks to several months (Driancourt, 2001). In monovulatory species such as cattle, follicular growth occurs in a wave-like pattern, with the number and length of waves depending on multiple factors such as age and breed. Follicular waves begin to develop as early as 2 weeks of age in beef heifers, and the number of follicles in each wave increases as females mature to puberty (Evans et al., 1994). In the wave-like pattern of follicle growth, critical events govern growth and development of follicles during the estrous cycle of beef cattle which include: recruitment, selection, dominance, and ovulation or atresia.

Before follicle wave growth can occur, recruitment of a cohort of small antral follicles from the primordial pool occurs. The recruitment phase lasts 1-3 days, recruiting only gonadotropin dependent follicles (Driancourt, 2001). Recruitment is consistent with an FSH surge 1-2 days earlier (Adams et al., 1992). After a wave of 5-30 follicles emerges, only a select few follicles commence to the next phase of follicular wave development as progesterone begins suppressing gonadotropins needed to support

growth. In order to continue to the next phase, a follicle must begin to support its own growth by increasing estrogen production and inhibit growth of all other follicles (Savio et al., 1988). In cattle, 1-2 follicles progress out of the recruited cohort towards dominance and ovulation. Ginther et al. (1996) observed that the largest follicle in the cohort is generally the one selected for future dominance, due to its earlier development of LH receptors on the granulosa cells and earlier growth divergence. The remaining follicles of the cohort that were not selected are regressed by atresia due to regression of LH and FSH receptors. The dominant follicle will then diverge further from its secondary follicle cohort with accumulation of more gonadotropins and hormones (Fortune, 2003). Thus, the dominant follicle becomes the ovulatory follicle and controls the growth of all other subsidiary antral follicles remaining in the wave.

2.4.4 Follicular wave characteristics

As stated previously, the number of follicular waves that an animal undergoes during a normal estrus cycle is different between species. For example *Bos taurus* cattle have 2-3 follicular waves (Savio et al., 1988; Ginther et al., 1989); while *Bos indicus* can have 3-5 waves per cycle (Sartori and Barros, 2011). Cows that have two follicular waves have longer wavelengths, undergo longer periods of follicular growth per wave, and ovulate larger, more mature follicles compared to 3-wave cows (Townson et al., 2002). In 2-wave cows, the first wave will emerge on d 0 (time of ovulation) and the second wave will emerge on d 10 of the cycle, while emergence of waves in 3-wave cows occurs on d 0, d 9, and d 16 (Ginther et al., 1989). The largest follicle from the first wave (2-wave cows) and first two waves (3-wave cows) will undergo atresia and not ovulate during a

normal cycle due to progesterone concentrations inhibiting estrogen and the LH surge required for ovulation. Therefore, the dominant follicle will arise from the second wave or third wave (2 and 3 wave cows, respectively) and will become the preovulatory follicle when negative feedback of progesterone is removed allowing ovulation to occur (Ginther et al., 1989; Adams et al., 2008).

2.5 Concepts of nutrition

In the beef industry, one of the most important factors impacting reproductive success is nutrition. Nutrition influences reproductive functions from follicular development to ovulation, hormone production, fertilization and ultimately pregnancy (Short and Adams, 1988); therefore, dietary formulations can utilize nutrients including energy and protein which fulfill nutrient requirements of beef females specific to their stage of production (gestation, lactation, or maintenance). However, if protein and energy requirements are not met, biological processes are likely disrupted which have negative effects on reproductive parameters.

2.5.1 Nutrient restriction

Restricting dietary nutrients prepartum have been shown to lengthen postpartum interval, decrease conception rates, and subsequently, pregnancy rates of beef cows as reviewed by Randel (1990). Cows with restricted energy intake are likely to lose body weight (BW) and body condition score (BCS), induced by a negative energy balance (Perry et al., 1991). Dziuk and Bellows (1983) and Richards et al. (1986) suggest calving cows at a BCS of ≥ 5 (on a 1-9 scale; Wagner et al., 1988) allows the female enough

energy reserves to support milk production, maintenance and subsequent reproductive functions. Nutrient restriction and the resultant negative energy state suppresses the function of the hypothalamic-pituitary-gonadal axis by decreasing the pulsatile release of LH into circulation, which may interrupt proper ovarian activity (Short and Adams, 1988).

More specifically, protein is a key nutrient that may often be overlooked in ration formulation, especially in cow-calf production settings where low quality forages are commonly consumed. Restricting protein intake of cows reduces first service conception rates and pregnancy rates compared to cows fed adequate protein intake. In a study by Sasser et al. (1989), cows fed deficient crude protein (CP; 0.32 kg/d) had first service conception rates of 25% and season pregnancy rate of 32%, which was significantly reduced compared to conception rates of their counterparts fed adequate CP (0.96 kg/d; 75% first conception and 74% overall pregnancy rates).

2.5.2 Dietary crude protein

With continued expansion in the ethanol industry, meeting protein requirements is not as expensive as it once was due to the growing supply of protein supplements available through ethanol coproducts. However, as the processes change, there is variation in the coproducts available (Lundy and Loy, 2014). Thus all protein supplements are not created equally and may differ compositionally according to rumen degradability and feeding value.

Dietary CP is composed of degradable and undegradable protein; defined by the amount of protein available for degradation into AA and peptides by rumen microbes.

Pichard and Van Soest (1977) described a series of fractions which make up dietary protein including fractions A, B1, B2 and C, based on the degradability of the dietary protein source. Fractions A and B1 are rapidly degraded in the rumen, while B2 is more slowly degraded and C is insoluble in the rumen. Many coproducts such as corn distillers grains and brewers grains, are marketed as dried products, and thus are exposed to heat which can cause a Maillard reaction to occur. The Maillard reaction is a process that occurs with intense heat exposure which affects the aldehyde and amino group of a protein, thus decreasing the digestibility and rendering it less soluble (Ferguson, 1975). However, generally these coproducts do not undergo severe heat damage that will completely bind proteins; thus, protein fractions are still available for digestion and potential absorption in the small intestine (Waldo and Goering, 1979).

2.5.3 Degradable protein

Protein sources that can be broken down in the rumen to be absorbed as amino acids (AA) for microbial protein synthesis, include urea (also known as non-protein nitrogen) and rumen degradable protein (RDP). Solubility of degradable protein can range anywhere from 100% degradable (urea) to less than 25% (blood meal), along with intermediate RDP products such as soybean meal (SBM; 75%; NRC, 2000). The soluble fraction of RDP will be metabolized into amino acids and peptides by protease enzymes, before being utilized as energy for rumen microorganisms (Butler, 1998). The amino acids can then be further broken down into organic acids, carbon dioxide, ammonia, and VFA (Staples et al., 1993). Depending on energy availability in the rumen, AA can be incorporated into microbial protein or deaminated into VFAs (Bach et al., 2005). Non-

protein nitrogen contributes to the N pool and can be incorporated into ammonia, DNA, RNA, AA or small peptides and is also used for microbial protein synthesis (Bach et al., 2005). If more ammonia is present than microorganisms can use, it is absorbed through the rumen wall into to the portal vein, where it becomes detoxified into urea by the liver. From the liver, the urea can be released into blood circulation for recycling or excretion. Ruminants have the unique capability of recycling N through saliva to return to the rumen for breakdown to ammonia; however, when there is adequate urea in the rumen, it is secreted in the urine and milk (Staples et al., 1993).

2.5.4 Undegradable protein

After the rumen degradable portion of a protein source is broken down by the rumen microbes, the remaining portion of feed protein that is undegradable to the rumen will pass to the intestine. This undegraded/ remaining protein is primarily termed rumen undegradable protein (RUP). Undegradable protein is passed to the small intestine where it is broken down by peptidase enzymes into peptides and AA. Once RUP is broken down, the resulting AA and peptide are available for absorption though the intestinal wall and utilized directly by the animal to support milk production, growth, fetus development, etc. (Ruminant Nitrogen Usage, 1985). In addition, approximately 50-80% of the protein escaping the rumen is microbial crude protein (MCP; discussed later) is synthesized in the rumen. Fractions of RUP and MCP that remain undigested or incapable of being absorbed in the intestine into blood circulation, is passed on through the remainder of the digestive tract.

2.5.5 Metabolizable protein

Overall, the goal of protein supplementation is to supply adequate RDP to feed the microbial population of the rumen and adequate MCP and RUP to support production and life processes of the animal. This is commonly accomplished by formulating diets to meet metabolizable protein (MP) requirements. According to the NRC (2000), MP is defined as the amount of protein that becomes absorbed in the small intestine. The composition of MP includes microbial crude protein (MCP), undegradable protein and bypass protein. Microbial crude protein is synthesized in the rumen from peptides, AA, and ammonia being joined by peptide bonds. A large portion of MCP flows to the small intestine where the pH and enzymes are capable of digesting MCP for absorption (Bach et al., 2005). As discussed previously, RUP reaches the small intestine (SI) by resisting degradation by microbes. Bypass protein escapes the rumen due to inadequate exposure to microbes (Ruminant Nitrogen Usage, 1985).

The quantity of MP passing to the SI is highly affected by dietary factors such as DMI and type of feedstuff (forage or concentrate), which in turn affect passage rate and pH of the rumen. Cows with low DMI have slower rumen turnover, allowing a larger degree of protein to be degraded in the rumen. Whereas with high DMI, rumen turnover occurs more frequently; thus, less protein is degraded in the rumen and passage rate to the SI increases. Furthermore, at more acidic pH, bacteria N flow to the intestine increases (Hoover and Stokes, 1991). In order to increase MP reaching the SI, additional RUP may be included in the diet. Therefore, managing type of protein supplementation is a more efficient method to increase the amount of protein reaching the SI than attempting to manipulate MCP passage rate (Ruminant Nitrogen Usage, 1985; Canfield et al., 1990).

2.5.6 Amino acids

Traditionally AA have been defined as essential AA (EAA), of which the body cannot synthesize and must be obtained from the diet, or nonessential AA which the body is capable of producing (NRC, 2000). There are 20 AA , with 10 being EAA essential for maintenance and growth which include: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. Ruminants and pigs are capable of synthesizing arginine through the urea cycle, but it remains an EAA for other species such as birds and fish (Boisen et al., 2000). Nonessential AA include cystine, tyrosine, alanine, asparagine, glutamine, serine, glycine, proline, aspartate and glutamate, which are all produced during glucose oxidation (Boisen et al., 2000).

The efficiency of protein utilization is measured by the composition of AA entering the small intestine. Depending on type of protein supplement, the amount of metabolizable AA that will be absorbed can also be quite different (Mjoun et al., 2010a; 2010b). Analysis of traditional dry distillers grains with solubles (DDGS) and reduced fat DDGS revealed similar total AA compared to SBM supplement. However, the quantity of each individual AA contributing to the total was shifted depending on supplement, as were the digestibility and absorbable fractions (Mjoun et al., 2010a). Nevertheless, lactating dairy cows consuming these three supplements differing in AA composition had similar milk production despite different arterial circulation of total AA concentrations (Mjoun et al., 2010b). While Grings et al. (1992) observed greater milk production after increasing inclusion of CP and RUP, this could have been due to enhanced branched-chain AA (BCAA) uptake. However, as Mjoun et al (2010b) also observed greater

BCAA extraction, negation of increased milk production may have been mediated by similar total AA concentrations.

2.5.7 Plasma urea nitrogen

Alterations in the progression of events towards successful reproduction in dairy and beef cows is most likely initiated by increased plasma urea N (PUN), serum or milk urea N concentrations. Urea N is a product of protein catabolism and is small enough to become incorporated into a variety of body cells, tissues and fluids which can lead to impaired reproduction (Butler et al., 1998). Urea N can be produced from detoxification of ammonia from RDP or deamination of AA after excess RUP consumption. When the liver converts these products to urea, it is capable of being released into circulation, recycled in the rumen or excreted in urine and milk. Both forms of CP contribute to urea formation which comes at an energetic cost to the female (Staples et al., 1993). Compared to excess RDP, excess RUP supplementation was shown to reduce PUN (Carroll et al., 1994; McCormick et al., 1999). However, when excess RUP was supplemented, concentrations of PUN were increased compared to baseline concentrations (Gunn et al., 2014b; 2014c) but not the extent of excess RDP diets (17 and 10 mg/dL vs. 19 mg/dL). Thus regardless of source, excess CP may provide energetic expenditure on the animal by altering PUN which may potentially elicit negative effects on reproduction.

Still, while indulging in excess protein has shown to be undesirable, there is evidence that long term supplementation can allow cows to adapt to elevated urea concentrations, which resulted in no negative effects on embryo development (Dawunda

et al., 2004; Gath et al., 2012). Excess CP may potentially be used for other reproductive parameters including increasing energy status and milk production (McCormick et al., 1999). Therefore, excess CP seems to be more likely to adversely affect reproduction if energy is in a deficit, but when metabolizable energy requirements are met, excess CP may be beneficial to reproductive functions.

2.6 Protein and reproduction

The effects of nutrient deficiency on ruminant reproductive functions have been thoroughly investigated and reviewed (Short and Adams, 1988; Randel et al., 1990; Perry et al., 1991; Robinson et al., 2006). In addition, protein requirements have been established for growth and maintenance (NRC, 2000); however, protein requirements for reproduction have not been fully characterized and the impacts of excess supplementation on reproduction are yet to be established. Furthermore, studies evaluating the effects of excess protein supplementation on ovarian function and consequently reproductive functions have elicited inconsistent results.

2.6.1 Excess protein supplementation

Excess protein supplementation has been shown to differentially affect reproductive function in dairy and beef cattle. Two common theories of how extra CP negatively impacts reproduction in dairy cattle exist. The first theory relates to the ability of urea to interrupt signaling between the hypothalamus and ovary by decreasing gonadotropin release and subsequent hormone responses (Jordan and Swanson, 1979a). The second theory postulates that excess CP consumption results in an abundance of

ammonia and urea in the reproductive tract, changing the uterine secretions (Jordan et al., 1983) and pH (Elrod and Butler, 1993) which alter the uterine environment to be less favorable for embryo development and fertilization. Nevertheless, not all researchers have reported a negative relationship between excess CP and reproduction of dairy and beef cows (Howard et al., 1987; Rusche et al., 1993; Lents et al., 2008).

2.6.2 Excess degradable protein

Lactating dairy cows have commonly been the subject of interest when analyzing how overfeeding highly degradable protein effects reproduction, as increasing RDP is a common practice to stimulate greater milk production (Butler et al., 1981). However, while greater RDP intake maximizes milk production, reproductive efficiency is usually sacrificed. Jordan and Swanson (1979a) observed a negative correlation between excess RDP and reproductive efficiency (services per conception and days open) when CP increased from 12%, 16% to 19% in the diet. Furthermore, additional dairy literature has noted a consistent negative relationship between conception rates and urea N concentrations at or above 19 mg/dL (Ferguson et al., 1988; Canfield et al., 1990; Butler et al., 1996; Ferguson et al., 1993). Nevertheless, other studies have reported when dairy cows in early lactation were supplemented with 20% CP, circulating urea N exceeded 24 mg/dL yet resulted in no negative effects on overall pregnancy rates (Howard et al., 1987; Carroll et al., 1988).

In multiparous beef cows supplemented with either 1.2 kg/d or 2.5 kg/d of a 42% CP soybean meal based supplement, Lents et al. (2008) found no difference in artificial insemination (AI) pregnancy rates of postpartum lactating cows. In addition, when

primiparous beef heifers were supplemented with soybean meal at 100% or 150% of CP requirements, PUN concentrations were greater with more RDP intake but no differences in conception rate existed (Rusche et al., 1993). Furthermore, excess RDP seem to not suppress pregnancy of beef females as much as dairy females; however, as both females are under lactation stress near breeding, any extra energy expended to metabolize excess protein may cause females to fall into a negative metabolic energy status reducing the amount of energy available for reproductive functions (Staples et al., 1993).

As lactating dairy cows consume more dry matter (DM) than non-lactating cattle in order to support milk production (NRC, 1989), this substantial dry matter intake (DMI) increases liver blood flow. By increasing blood flow, metabolism of steroid hormones (estrogen and progesterone; Sangsritavong et al., 2002) also increases. Thus, DMI may reduce circulating levels of these hormones, ultimately affecting the feedback mechanisms driving successful reproductive functions. Jordan and Swanson (1979b) further investigated reduced progesterone concentrations in lactating dairy cows consuming excess RDP and discovered potential competitive action of urea interrupting binding of LH to LH receptors on the CL (Haour and Saxena, 1974). Furthermore, hormone production may be mediated by stage of production as lactating cows had reduced concentrations of progesterone after excess RDP consumption (Jordan and Swanson, 1979b; Sonderman and Larson, 1989; Staples et al., 1993), but progesterone concentrations of non-lactating cows were not affected by amount of RDP in the diet (Elrod and Butler, 1993; Garcia-Bojalil et al., 1994).

Excess RDP has also been found to alter uterine secretions during the estrous cycle in dairy cattle (Jordan et al., 1983; Elrod and Butler, 1993; Elrod et al., 1993).

Normal uterine pH during the estrous cycle ranges from 7 during the luteal phase to 6.7 during estrus (Perry and Perry, 2008). When cattle are fed excess RDP, urea may decrease uterine pH which can be toxic to embryos (Ferguson et al., 1988) and suppress maternal recognition of pregnancy due to altering uterine secretions of Mg, K, and P (Jordan et al., 1983). Furthermore, sperm transport and capacitation is driven by uterine pH returning to 7 after estrus (Florman et al., 1989); therefore, excess RDP may interrupt sperm transport and impair sperm from accomplishing fertilization.

2.6.3 Excess undegradable protein

While excess dietary RDP is commonly linked to suppressed reproductive function, excess RUP supplementation has been reported to have more positive effects on reproduction by shortening postpartum interval (Figueroa et al., 1992; Sinclair et al., 1994) and improving conception rates in dairy cows (Armstrong et al., 1990; Bruckental et al., 1989). In addition, studies which compared excess supplementation of highly undegradable to degradable feedstuffs, greater RUP supplementation increased first service conception rate in beef heifers (Wiley et al., 1991; Martin et al., 2007) and overall pregnancy rates in dairy cows (McCormick et al., 1999). When gestating beef heifers (Gunn et al., 2014b) and primiparous beef heifers (Rusche et al., 1993) were supplemented with 100% or 150% RUP, similar AI and overall breeding season pregnancy rates were observed. However, female progeny of beef heifers supplemented with excess DDGS (Gunn et al., 2014b) had greater AI pregnancy rates than progeny of females only supplemented with 100% CP requirements (Gunn et al., 2015).

Since AA and peptides from degraded RUP is absorbed in the intestine and is readily available to the ruminant, excess RUP has shown to stimulate the pancreas to increase insulin production (Sletmoen-Olson et al., 2000; Schroeder et al., 2005). Insulin affects ovarian tissues by enhancing LH receptor synthesis and actions of the pituitary through these receptors (Butler and Canfield, 1989). Kane et al. (2002) suggested that undegraded protein works to improve reproduction by mediating LH and FSH production. Although Wiley et al. (1991) and Rusche et al. (1993) observed no difference in insulin or LH parameters between beef cows fed excess RUP or RDP, perhaps through a coupling of mechanisms, greater RUP may have the potential to increase insulin which may enhance gonadotropin synthesis, and potentially improve reproductive efficiency.

2.6.4 Excess protein and ovarian function

As previously discussed, the association between protein and overall fertility can elicit various effects on reproduction depending on source and amount of protein. However, the effects of excess protein supplementation on beef cattle ovarian function specifically have not been fully investigated. Excess urea has shown to not affect ovarian parameters of CL development, embryo development or embryo recovery rate in beef heifers (Gath et al., 2012), which may mean that excess protein effects early oocyte development rather than blastocyst formation. Rhoads et al. (2006) observed that high PUN affected oocytes before d 7 of pregnancy, but did not determine the exact time when oocytes were most susceptible to urea. When Santos et al. (2009) incubated oocytes in urea, cleavage rates and blastocyst development was more reduced in the high PUN (> 16 mg/dL) group than was observed at lower concentrations (< 16 mg/dL). Elevated PUN

seems to affect health of preovulatory oocytes and follicles between 4 and 8 mm more than post ovulation embryo development (Sinclair et al., 2000). This could potentially be due to urea altering growth and metabolism of the granulosa cells of follicles, by interfering with gonadotropins (Rooke et al., 2004).

Lents et al. (2008) was the first to observe that greater protein supplementation enhanced dominant follicle size in postpartum multiparous beef cows. Follicle diameter deviation between greater and lesser protein supplementation may have been due to greater protein intake increasing insulin like growth factor-1 (IGF-1), which may act on ovarian function via the hypothalamo-pituitary-ovarian axis (Diskin et al., 2003). Most recently, excess protein from DDGS supplemented to gestating and lactating beef heifers, enhanced ovarian function compared to heifers on a control silage TMR diet, as dominant and secondary follicle diameters, as well as wavelength were greater in high protein intake heifers (Gunn et al., 2014b).

Because the CP supplement used by Gunn et al. (2014b) contributed both protein and fat to the diet (sourced from DDGS), the dietary component driving these effects on follicular growth could not be fully attributed to protein. However, when non-lactating cows were supplemented with a higher RUP, lesser fat supplement (corn gluten meal: CGM), enhanced growth of the preovulaotory follicle, antral follicle count, ovulatory follicle size, and estradiol concentrations were observed (Gunn et al., 2014c). Collectively these studies indicate the excess nutrients (fat and protein) are beneficial to follicle growth. More specifically, excess RUP seems to play an important role in follicular growth as highly RUP concentrated, lesser fat supplements presented similar results as traditional protein supplements when consumed by beef cows.

2.6.5 AA and reproduction

While essential and nonessential AA have roles throughout the body, the specific interactions between AA and reproductive system have not been highly explored. Nevertheless, some connections are beginning to be made between some AA and reproductive functions. Leucine, a branched chain AA, activates the mTOR protein synthesis pathway (Greiwe et al 2001; Bazer et al., 2009; Herman et al., 2010) which has been observed to increase proliferation of cultured rat granulosa cells and increased follicle growth (Yu et al., 2011), as well as drive mechanisms associated with LH and bovine luteal tissue synthesis (Zhang et al., 2011). Furthermore, in cultured rabbit oocytes, proline and glutamine, when added separately promoted oocyte maturation (Bae and Foote, 1975). Moreover, arginine plays a role in survival, growth and development of the embryo in sheep, pigs and rats (Wu et al., 2009). In addition, arginine synthesizes nitric oxide which stimulates vascular functions, and proliferation and differentiation of cells developing the fetus and placenta (Wu & Morris, 1998; Wu et al., 2004) and increases growth hormone and LH secretion in dairy cattle (Chew et al., 1984).

2.7 Statement of the problem

With the continued growth of the ethanol industry and volatile commodity prices in recent years, beef producers have been utilizing a wide variety of feedstuffs to meet nutrient needs of their cattle while maintaining profitability. In the cow-calf sector of the beef industry, pairing crude protein coproducts with low quality forage or corn stalks to winter cattle is a common practice. While this practice is commonly cost-effective, over

supplementation of dietary protein is likely. Moreover, with the emergence of fractionated ethanol coproducts which are rich in protein and less concentrated in fat than traditional coproducts, determining how excess CP may impact beef cow reproduction warrants investigation.

Excess crude protein supplementation in dairy cattle has commonly resulted in poor fertility associated with greater urea N in circulation which is linked to altered uterine environments, impairing fertilization and embryo survival. However, depending on the rumen degradability of the protein supplemented, differential effects on reproduction have been observed. More specifically, excess dietary protein from a moderately rumen undegradable protein source has been reported to positively impact ovarian follicular development in beef cows. Furthermore, the question arises of whether type of protein, amount of protein or synergistic effect of both should be utilized to maximize ovarian reproductive parameters.

The overall goals of the experiments included in this thesis are to determine the impact of feeding excess dietary protein from feedstuffs differing in rumen degradability, as well as assess differences between amounts of excess protein, on ovarian function of beef cows consuming low quality forage. From these goals, the biological mechanisms of which excess dietary protein impacts ovarian function may be elucidated, by which may be useful in understanding ways supplementation can be utilized to alter reproductive function and overall fertility of beef cows.

CHAPTER 3.

EFFECTS OF EXCESS DIETARY METABOLIZABLE PROTEIN FROM CORN GLUTEN MEAL OR SOYBEAN MEAL ON OVARIAN FUNCTION AND CIRCULATING AMINO ACID CONCENTRATIONS OF BEEF COWS CONSUMING LOW QUALITY FORAGE

3.1 ABSTRACT

The objective of this experiment was to determine the effects of feeding excess MP from feedstuffs differing in rumen degradability on ovulatory follicular dynamics, subsequent corpus luteum (CL) development, steroid hormone production and circulating AA in beef cows. Non-pregnant, non-lactating Angus and Angus-Simmental mature beef cows (n = 18) were stratified by age, BCS and BW to 1 of 2 isocaloric, isonitrogenous diets of ad libitum corn stalks and supplemented with corn gluten meal (moderate RUP; CGM) or soybean meal (low RUP; SBM) at 150% of MP requirements for 58 d. After a 20-d supplement adaptation period, cows were synchronized for ovulation using the 5-d CO-Synch + CIDR protocol. Ten days after synchronization completion, GnRH was administered to reset ovarian follicular growth. Starting at GnRH administration and daily thereafter until spontaneous ovulation, transrectal ultrasonography was performed to diagram ovarian follicular growth, and blood samples were collected for hormone, metabolite and AA analyses. Seven days after visual detection of estrus, CL size was determined via ultrasound and supplementation ended. Cows were then offered ad libitum corn stalks for 18-d to allow plasma urea N (PUN) to return to baseline. Data

were analyzed using the MIXED procedures of SAS. As designed, cow BW and BCS were not different ($P \geq 0.55$). Ovulatory follicular wavelength, antral follicle count, ovulatory follicle size at dominance and duration of dominance were not different ($P > 0.13$) between treatments. Cows supplemented CGM had greater post-dominance ovulatory follicle growth, larger dominant follicles at spontaneous luteolysis, shorter proestrus, and larger ovulatory follicles ($P \leq 0.03$) than SBM cows. No differences ($P \geq 0.11$) in peak estradiol, ratio of estradiol to ovulatory follicle volume, or PUN were observed. While CL volume and the ratio of progesterone to CL volume were not affected by treatment ($P \geq 0.24$), CGM treated cows tended to have decreased ($P = 0.07$) circulating progesterone 7 d post-estrus compared to SBM cows. Although total circulating plasma AA concentration did not differ ($P = 0.37$) between treatments, as a percent of total AA CGM cows had greater circulating leucine, phenylalanine and proline ($P \leq 0.02$) than SBM cows. In summary, these data illustrate that excess MP when supplemented to cows consuming a low quality forage may differentially impact ovarian function depending on ruminal degradability of the protein source.

3.2 INTRODUCTION

Distillers grains (DG) provide a concentrated package of protein and energy, which when paired with low quality forages constitute nutrient rich diets (Klopfenstein et al., 2008) that have been shown to improve performance and pregnancy rates in yearling heifers (Engel et al., 2008) and cows (Gunn et al., 2014a). However, these reproductive improvements may have been due to additional dietary unsaturated fat in these

coproducts, which has been reported to improve ovarian function (Robinson et al., 2002) and pregnancy rates in dairy cattle (Staples et al., 1998).

With the emergence of fractionated ethanol coproducts that are rich in protein and less concentrated in fat, determining how excess dietary CP in the absence of excess fat impacts reproduction warrants investigation. Previous research in dairy cattle has reported a link between excess CP and suppressed fertility (Elrod and Butler, 1993; Butler et al., 1996). However, the aforementioned data were derived from dairy cows consuming feedstuffs with elevated RDP fractions. At present there has been little research conducted on the effects of excess CP on ovarian function of beef cows until recently when excess CP diets rich in RUP were reported to positively impact ovulatory follicle growth (Gunn et al., 2014b; 2014c).

Thus, the question arises whether source of protein and rumen degradability of that protein, when fed in excess, differentially impacts ovarian function of beef cows. The objective of this study was to evaluate the effects of feeding 150% of MP requirements with supplements having a moderate (corn gluten meal) or low (soybean meal) RUP fraction, on ovarian function and AA concentrations of beef cows nearing ovulation. We hypothesized that excess MP from a moderate RUP source would improve ovulatory follicle growth, as well as increase circulating hormone and AA concentrations compared to excess MP from a feedstuff with less RUP.

3.3 MATERIALS AND METHODS

3.3.1 ***Animals and diets.*** All cows were handled in accordance with procedures and protocols approved by the Iowa State University Institutional Animal Care and Use

Committee. Research was conducted at Iowa State University Zumwalt Station Research Center in Ames, Iowa from January 2014 to March 2014. To study the effects of excess dietary MP on ovarian function, non-pregnant, non-lactating, Angus and Angus-Simmental multiparous beef cows were allocated by age, BW, and BCS (n = 18; age = 6.4 ± 3.4 yr; BW = 502 ± 78 kg; BCS = 4.5 ± 0.4 [1 = emaciated, 9 = obese; Wagner et al., 1988]), to 1 of 2 isocaloric, isonitrogenous supplements, differing in degradability of protein (Table 3.1). Cows were allowed ad libitum access to processed corn stalks (6% CP and 80% NDF) and fed either 1) corn gluten meal-based (**CGM**) or 2) soybean meal-based (**SBM**) supplement, formulated to target an ADG of 0.45 kg/d and equal 150% of MP requirements (NRC, 2000). Supplement was provided for the first 58 d of the study and ad libitum corn stalks were offered for a total of 76 d (Figure 3.1). Diet formulation was based on feedstuff analysis prior to study initiation (Dairyland Laboratories Inc., Arcadia, WI).

Individual supplementation occurred at 0800 h daily inside a facility where 10 side-by-side stanchions were located. All cows from one treatment were restrained in stanchions and delivered individual supplement. Cows remained in stanchions for < 1 hr until supplement was consumed and daily measurements and observations had been recorded. After one treatment group consumed supplement, cows were released from stanchions and the next treatment group was brought into the stanchions for individual supplement delivery.

3.3.2 ***Performance characterization.*** Initial BCS was taken on the day prior to treatment initiation, and initial BW was the average of BW taken on the day prior to and the first day of the study prior to supplement delivery. Subsequent BW and BCS were

taken monthly throughout the study, with all BW averaged on 2 consecutive days prior to supplement deliveries to account for potential differences in gut fill. To minimize differences in gut fill, animals had restricted access to corn stalks for 12-h prior to mornings in which BW were recorded. Body condition scores were the average of scores assessed by the same 2 trained investigators at each time point. Final BW and BCS were taken at the end of supplementation and prior to the 18-d period where ad libitum corn stalks were the only allotted feedstuff.

3.3.3 *Experimental Design*

3.3.3.1 ***Pre-synchronization.*** The experimental design is illustrated in Figure 3.1. Following a 20-d dietary adaptation period, cows were synchronized for ovulation using the 5-d CO-Synch + CIDR protocol. At synchronization initiation cows were administered 100 µg GnRH (Cystorelin, Merial Limited, Duluth, GA) and received an intravaginal progesterone insert (**CIDR**, Zoetis Animal Health, New York, NY). Five days later, the CIDR was removed, 25 mg $PGF_{2\alpha}$ (Lutalyse, Zoetis Animal Health, New York, NY) was administered and an Estrotect^{TM} heat detection aid (Rockway Inc., Spring Valley, WI) was placed on the tailhead of each cow. A second 25-mg dose of $PGF_{2\alpha}$ was administered 8 h after CIDR removal. Estrus detection was performed for 72 h following CIDR removal by trained personnel. Cow were monitored for visual estrus twice daily for 30 min each at 0630 h and 1700 h. Seventy-two hours after CIDR removal and initial $PGF_{2\alpha}$ injection, all cows received 100 µg GnRH, and heat detection aid activity was recorded and removed.

3.3.3.2 ***Ovulatory follicle wave characterization***. Ten days after completion of the synchronization protocol, 100 µg GnRH was administered to force development of a new

ovarian follicular wave. Starting at GnRH administration and daily thereafter at 0800 h, cows were subject to transrectal ultrasonography (Ibex^{TM} Portable Ultrasound, variable MHz linear array transducer, E.I. Medical Imaging, Loveland, CO) for characterization of the ovulatory follicular wave. Ultrasound examinations were performed by the same investigator throughout, and estrus detection was visually observed twice daily at 0630 h and 1700 h by trained personal with the aid of heat detection patches. The location and size of all antral follicles ≥ 3 mm in diameter were recorded by drawing representative sketches of each ovary. Follicles were measured using the caliper function of the ultrasound to determine the diameter of a given follicle using the average of the greatest cross-sectional perpendicular measurements.

The dominant follicle was documented as the largest growing follicle of the wave and the secondary follicle was the second largest growing follicle in the same wave. Day of dominance was categorized as the day on which the largest growing follicle was at least 8 mm in diameter and at least 1 mm greater in diameter than any other growing follicle of the same wave. The duration of dominance was defined as the number of days from attainment of dominance until ovulation. Daily ultrasonography continued until ovulation was observed as confirmed by disappearance of the dominant follicle when precluded by visual estrus expression.

Once estrus was detected and subsequent ovulation was confirmed, ultrasound measurements ceased until 7 d post-estrus, when ultrasound was performed to measure the CL at the site of ovulation. Total CL volume was calculated using the caliper function of the ultrasound and formula for a rotary ellipsoid V= 4/3 Π Iab^2 (where a = longitudinal axis and b = transverse axis). If the CL contained a lacuna, the volume of the lacuna was

also calculated using the rotary ellipsoid equation and subtracted from the total CL volume to determine total volume of luteal tissue.

Day of spontaneous luteolysis during the ovulatory follicle wave was retrospectively determined to be the first day on which circulating progesterone concentrations were ≤ 1 ng/mL via RIA. This allowed for dominant follicle size at luteolysis and follicle growth from luteolysis to ovulation to be determined. After conclusion of the ultrasound period, day of ovulatory follicle wave emergence was tracked retrospectively to a cohort of follicles from which the dominant follicle originated (3-4 mm in diameter). Wavelength was defined as the number of days from emergence to ovulation. Antral follicle count (**AFC**) was totaled across both ovaries daily and averaged for the entirety of the wavelength to determine the daily AFC average.

3.3.4 ***Plasma analyses.*** Starting at study initiation, coccygeal blood samples were collected every other day until the beginning of ultrasound period for evaluation of PUN concentrations. During the ultrasound period until final CL measurement, blood samples were collected daily for determination of circulating PUN, progesterone, and estradiol-17β concentrations. One blood sample was collected 8-d after onset of the ovulatory follicular wave to determine circulating AA concentrations around the time of ovulation. After CL measurement and protein supplement termination, blood sample collection continued twice weekly for 18 d to characterize the return of circulating PUN concentrations to baseline. Blood samples were collected via coccygeal venipuncture in 6 mL EDTA vacutainer (10.8 mg EDTA; BD Vacutainer^{TM}; Becton, Dickinson and Co., Franklin Lakes, NJ) and immediately placed on ice. Samples were centrifuged at 1,750 x

g for 25 minutes at 4° C. Plasma was recovered and aliquoted into 5 mL polystyrene tubes and frozen at -20° C until hormone, metabolite, and AA analyses were conducted.

Plasma samples collected on the day of luteolysis through the day of ovulation were analyzed to determine peak circulating concentrations of estradiol-17β via RIA at South Dakota State University using the methodology described by Perry and Perry (2008). Across 2 assays, the average intra-assay CV was 4.4% and the inter-assay CV for a pooled serum sample containing 8 ± 0.8 pg/mL was 10%. The average sensitivity across assays was 0.45 pg/mL (95% confidence interval). Plasma collected during the ultrasound period was analyzed for progesterone concentration to determine when luteolysis occurred, as well as to track function of the CL that resulted from spontaneous ovulation of the ovulatory follicle of interest. Progesterone concentration was analyzed via a commercially available RIA kit (Coat-A-Count, Siemens Healthcare Diagnostics Inc., Los Angeles, CA). Across 3 assays, the average intra-assay CV was 1.3%, with the inter-assay CVs for pooled samples containing 0.54 ng/mL and 5.69 ng/mL of progesterone, were 8.0% and 3.33%, respectively. The average sensitivity across assays was 0.15 ng/mL (95% confidence interval).

Plasma samples were analyzed for PUN with a commercially available kit (Urea Nitrogen Procedure No. 0580, Stanbio Laboratory, Boerne, TX). Samples were prepared and loaded into 96-well plates to be read at 520 nm in an Eon Microplate Spectrophotometer (BioTek Instruments, Inc., Winooski, VT). Across 24 assays, the average intra-assay CV was 4.7%, and the inter-assay CV for the pooled serum sample containing 3.94 mg/dL of urea nitrogen was 19.5%. Plasma collected 8-d after onset of the ovulatory follicular wave was analyzed for circulating AA concentrations at North

Dakota State University via Ultra Performance Liquid Chromatograph (Lemley et al., 2013).

3.3.5 ***Statistical analysis.*** Data were analyzed using the MIXED procedure of SAS 9.3 (SAS Inst. Inc., Cary, NC), with animal as the experimental unit and the main fixed effect of treatment. For all follicular wave characteristics, hormones, and metabolite analyses, cow age, individual initial BW and individual change in BW were included in the model as covariates. Follicular growth, PUN, and post-ovulatory progesterone concentrations were analyzed with the MIXED procedure of SAS for REPEATED measures. For all variables, the covariance structures of compound symmetry, heterogeneous compound symmetry, first order autoregressive, unstructured and ante-dependence were compared. The covariance structure with the smallest Bayesian information criterion result was used for the final analysis. The model included the fixed effects of treatment and day, as well as the treatment by day interaction. The SLICE function of SAS was used to generate simple effects within day.

Significance for all variables was declared at a P-value ≤ 0.05 and tendencies were identified at a P-value > 0.05 and ≤ 0.10. It should be noted that 2 cows from the SBM treatment and 1 cow from the CGM treatment failed to ovulate within 18-d of forced follicle turnover. Therefore, reproductive data from those 3 cows were excluded from the dataset; however, performance, PUN, and AA data from those cows remained in the data set and were included in the final analysis.

3.4 RESULTS

3.4.1 ***Performance characteristics.*** As designed, cow BW and BCS did not differ ($P \geq 0.55$; Table 3.2) at treatment initiation or at the conclusion of the supplementation period.

3.4.2 ***Ovulatory follicle wave characterization.*** Although there was no difference between follicle growth rate 6 d prior to ovulation ($P > 0.10$; Figure 3.2), ovulatory follicles of CGM cows experienced increased growth ($P < 0.01$; Table 3.3) after attainment of dominance and had larger follicles at luteolysis ($P = 0.03$) when compared to SBM cows. Furthermore, CGM cows had a shorter proestrus interval ($P < 0.01$), yet larger ($P = 0.03$) follicles at the time of ovulation than SBM cows. However, no differences ($P \geq 0.13$) in wavelength, AFC or CL volume were observed between treatments.

3.4.3 ***Plasma analyses.*** Peak estradiol concentrations at ovulation and the ratio of estradiol to ovualtory follicle size were not affected ($P \geq 0.24$, Table 3.4) by treatment. While no difference in progesterone concentrations existed from d 1 to 6 post-estrus ($P > 0.10$; Figure 3.3), on d 7 post-estrus circulating progesterone concentrations tended to be decreased ($P = 0.07$) in CGM compared to SBM cows. The ratio of circulating progesterone to CL volume 7 d post-estrus remained similar ($P = 0.24$) between treatments. However, there was a treatment × day interaction for PUN ($P = 0.02$; Figure 3.4), as cows supplemented with SBM had greater ($P = 0.05$) PUN on the last day of supplementation (d 19).

Total AA concentrations, as well as total essential AA, nonessential AA, glycogenic AA, and branched-chain AA concentrations did not differ ($P \geq 0.21$; Table

3.5) due to dietary treatment at d 8 after onset of the ovulatory follicular wave Total leucine and phenylalanine were greater ($P \leq 0.01$) and methionine tended to be greater ($P = 0.06$) in circulation of CGM than SBM supplemented cows. When analyzed as percent of total AA, excess MP supplementation with SBM increased ($P \leq 0.05$) concentrations of arginine, lysine, threonine, tryptophan and valine compared to CGM; while excess MP supplementation with CGM increased ($P \leq 0.05$) concentrations of leucine, phenylalanine, methionine, asparagine and proline compared to SBM.

3.5 DISCUSSION

3.5.1 ***General.*** The objective of this study was to evaluate the effects of excess MP supplementation from feedstuffs differing in rumen degradability on ovarian function of multiparous beef cows during the peri-ovulatory period. To minimize confounding factors of BW and BCS on ovarian function, isocaloric, isonitrogenous supplements were designed to maintain similar BW and BCS across treatments throughout the study. Data from this study indicate these goals were met, allowing for direct comparison between sources of excess dietary protein on ovarian function. Diets containing elevated amounts of RDP are largely accepted as likely to increase PUN when compared to diets containing increased RUP. While excess RUP can also be deaminated and may contribute to circulating urea, PUN concentrations were likely similar between treatments due the reduced total CP requirements of non-pregnant, non-lactating mature beef cows, resulting in a small amount of total CP fed per day. Nonetheless, the similarity in PUN concentrations between treatments, coupled with similar BW and BCS allows for the

unique opportunity to directly study the effects of pathways other than PUN which may alter ovarian function.

Previous studies conducted in dairy cattle have routinely associated excess dietary protein, resulting in PUN concentrations above 19 mg/dL, with decreased pregnancy rates (Ferguson et al., 1988; Elrod and Butler, 1993; Butler et al., 1996). Yet, as the previously mentioned studies utilized diets comprised of feedstuffs with substantial rumen degradable protein fractions, it is somewhat unclear if amount or source of dietary protein, or both, was the underlying cause of these associations. While few studies have explored the relationship between excess dietary protein and reproductive parameters in beef cattle, it has been widely assumed that reproductive impacts would be synonymous with those reported in the dairy literature. However, when CP was fed at 150% of NRC requirements to primiparous beef females, PUN concentrations were increased to 16 (Rusche et al., 1993) and 17 (Gunn et al., 2014b) mg/dL, but no negative effects on reproductive function or pregnancy rates were noted. Furthermore, in both of these studies, conception to artificial insemination was similar or improved in females consuming excess dietary CP when compared to those consuming 100% of CP requirements. Thus, as PUN concentrations in the current study did not reach 19 mg/dL after excess CP consumption from differing degradability feedstuffs, we were able to evaluate how source of excess protein affects ovarian function during the peri-ovulatory period without confounding PUN concentrations.

3.5.2 *Ovulatory follicle wave characteristics*

Length of estrous cycle and number of follicular waves during the cycle have been reported to affect beef cattle reproduction by influencing size and age of developing

follicles (Townson et al., 2002; Cushman et al., 2007). However, as we observed no differences in duration of dominance or wavelength between treatment groups, diameter deviation in follicle size post-attainment of dominance and larger ovulatory follicles in the CGM treatment was likely a direct effect of diet. Similarly, Gunn et al. (2014c) reported that excess RUP supplementation to non-lactating beef cows increased ovulatory follicle growth rate post wave emergence compared to cows consuming the lesser RUP diet.

It has been previously demonstrated that excess dietary CP supplementation yields larger dominant follicles in first-calf beef heifers (Gunn et al., 2014b), as well as in lactating cows (Lents et al., 2008). More specifically, when excess CP from corn gluten meal, a feedstuff moderate in RUP, was fed to non-lactating mature beef cows, larger ovulatory follicles were noted when compared to cows fed 100% of CP requirements (Gunn et al., 2014c). It should be noted that these previous studies examined the effects of increased dietary CP compared to a control that only met CP requirements. The current study compared similar excess dietary CP concentrations from differing sources and still noted differences in ovulatory follicle growth. Larger ovulatory follicles observed in this and previous studies in which RUP was fed in excess may result in greater embryo competency (Arlotto et al. 1996) and pregnancy success in beef heifers (Perry et al., 2007) and cows (Perry et al., 2005), compared to smaller follicles. However, there may be a threshold to the "larger is better" theory, as beef heifers ovulating follicles greater than 15.7 mm in diameter had a decreased probability of establishing a successful pregnancy (Perry et al., 2007). Ovulatory follicles of CGM cows in the present study are

near this threshold, and thus, further research may be warranted to determine ultimate effects of these diets on establishment of pregnancy.

Given that cows consuming SBM in the present study had smaller ovulatory follicles, it was not surprising that they also had a longer duration of proestrus, based on previous research demonstrating smaller dominant follicles tend to have lesser concentrations of estradiol driving growth (Vasconcelos et al., 2001). However, this was not the case in the present study as we did not observe differences in peak estradiol concentrations between treatments despite the smaller ovulatory follicles. We speculate that these smaller follicles may have initially been less responsive to gonadotropins, requiring more time to increase estradiol and reach ovulatory capacity, therefore lengthening proestrus (Perry et al., 2014). Conversely, Bridges et al (2010) reported that regardless of follicle size, beef cows exhibiting longer proestrus (2.2 d) resulted in greater preovulatory estradiol concentrations compared to short proestrus (1.2 d). However, we also failed to see a similar pattern between estradiol concentrations and proestrus length in the SBM treatment; therefore, neither follicle size, nor circulating estradiol concentrations seem to be controlling length of proestrus in the present study, which may indicate that excess protein was contributing to proestrus length through unknown mechanisms.

3.5.3 *Hormones, PUN and AA*

Hormones. As previously mentioned, large ovulatory follicles have been reported to contain more estrogen than their small counterparts (Vasconcelos et al., 2001; Atkins et al., 2013; Perry et al., 2014); potentially due to smaller follicles having fewer granulosa cells converting androgens to estrogen (Ireland & Roche, 1983; Vasconcelos et al., 2001).

However, similar to Lents et al. (2008) there was not a difference in preovulatory estradiol concentrations regardless of ovulatory follicle size after excess protein supplementation in the present study. Therefore, ovulatory follicles from both treatments may be of similar competency due to similar peak estradiol concentrations.

Several authors have reported concentrations of progesterone circulating 7 d post-estrus to be positively correlated with size of ovulatory follicle (Atkins et al., 2010) and subsequent CL size (Vasconcelos et al., 2001), where larger follicles with more granulosa cells result in greater CL volume and progesterone secretion (Jinks et al., 2012). However, as reported here a disconnect between ovulatory follicle volume, CL volume and progesterone concentrations after spontaneous ovulation in beef cows has been reported before (Perry et al.; 2005). It should be noted that although progesterone concentrations tended to be different in the current study, concentrations were still sufficient to support pregnancy (Busch et al., 2008). Yet this tendency for reduced progesterone in CGM supplemented cows may be of more concern in cows that are producing concentrations of progesterone that are borderline sufficient for pregnancy establishment, as greater progesterone concentrations are correlated with pregnancy success (Busch et al., 2008).

Plasma urea N. While PUN concentrations in the present study were numerically greater in cows supplemented with SBM, the dynamic changes in PUN that occurred throughout the supplementation period in both treatments despite consistent dietary delivery were not expected. Cows were consuming the same amount of protein each day, but for unknown reasons PUN concentrations began to fluctuate at onset of pre-synchronization and peaked between spontaneous ovulation and the final day of

treatment. Perhaps PUN started decreasing as a result of metabolic adaptation to the diets, but then increased during times of estrus if DMI of stalks decreased, creating a nitrogen energy imbalance (Gath et al., 2012). However, further research is warranted to determine how PUN concentrations may be affected by stage of the estrous cycle and if these shifts in metabolites have reproductive significance.

Amino acids. Research in dairy cows has evaluated protein degradability (Boisen et al., 2000; Mjoun et al., 2010a) and AA circulation post supplementation (Mjoun et al., 2010b); however, in what manner circulating AA concentrations may impact ovarian function remains widely unknown. In the present study, total AA, as well as total essential, and nonessential AA were not different between treatments, yet a shift in several individual circulating AA concentrations was noted. Kuhara et al. (1991) reported increased concentrations of metabolites and metabolic hormones in response to infusion of various AA in sheep, including effects of leucine, asparagine and phenylalanine and glycine on insulin, GH, and glucose respectively. The increased circulation of these metabolites and leucine may also function to increase growth rate of preovulatory follicles through pathways associated with IGF-1 (Ginther et al., 2000; Gutierrez et al., 2000) or mammalian target of rapamycin (mTOR) activation pathway, respectively (Herman et al., 2010).

Arginine has been shown to impact ovarian function via increasing hormone and LH secretion in dairy cattle and prepubertal ewes, respectively (Chew et al., 1984; Recabarren et al., 1996). However, arginine was only different between treatments when analyzed as a percent of AA. The combination of these points suggests that total circulating arginine as opposed to arginine ratios to other circulating AA, is more closely

associated with changes in reproductive function. Given similarities in BCS, BW, MP, and PUN in the current study, it is likely that differences noted in preovulatory follicle growth is a result of altered circulating AA profiles; however, further research is needed to fully understand which amino acids may have the largest impact not only on ovarian function, but general fertility in females.

3.5.4 *Conclusion*

Based on these data, source of CP when fed to 150% of MP requirements has differential impacts on ovarian function in mature beef cows. The larger ovulatory follicles observed from excess RUP supplementation was expected based on previous research; however, differences in progesterone circulation was not. Still, progesterone at these concentrations were plenty adequate to support reproductive functions in these cows, but may become a concern in cows with hormone imbalances. As ovarian function plays only a partial role in general fertility, further research is warranted to elucidate the exact mechanisms by which excess dietary RUP, when supplemented with low quality forage-based diets may impact ovarian function and fertility.

Table 3.1. Supplement provided to cows consuming ad-libitum corn stalks[2]

	Treatment[1]	
Item	SBM	CGM
Dry matter intake, kg/d		
Corn silage	0.26	0.46
Corn gluten meal[3]	--	1.04
Soybean meal[4]	1.33	--
Mineral	0.11	0.11
Calculated nutrient intake		
Total CP, kg/d	0.74	0.75
Total RUP, kg/d	0.26	0.45
NE_g, Mcal/d	2.14	2.3

[1]Treatment included ad libitum access to corn stalks combined with daily supplementation of either soybean meal (SBM) or corn gluten meal (CGM) to equal 150% of dietary MP requirements.
[2] Corn stalk nutrient analysis (% DM basis): 51% TDN; 6% CP; 50% ADF; 80% NDF.
[3] CGM Nutrient analysis (% DM basis): 47% CP; 62% RUP; 2.4% Fat.
[4] SBM Nutrient analysis (% DM basis): 50% CP; 36% RUP; 1.6% Fat.

Table 3.2. Effects of excess MP supplementation on BW and BCS

	Treatment[1]			
Item	SBM	CGM	SEM[2]	*P*-Value
BW, kg				
Initial	505	498	26.87	0.85
Final	545	537	28.82	0.85
BCS[3]				
Initial	4.43	4.54	0.13	0.55
Final	4.83	4.94	0.23	0.74

[1]Treatment included ad libitum access to corn stalks combined with daily supplementation of either soybean meal (SBM) or corn gluten meal (CGM) to equal 150% of dietary MP requirements.
[2] SEM: SBM n = 9; CGM n =9.
[3]BCS on scale of 1 to 9 (1= emaciated, 9= obese; Wagner et al, 1988).

Table 3.3. Effects of excess MP supplementation on ovulatory follicle wave and corpus luteum characteristics

	Treatment[1]			
Item	SBM	CGM	SEM[2]	*P*-Value
Ovulatory follicle size at dominance[3], mm	9.39	8.98	0.67	0.67
Dominance duration[4], d	5.78	6.95	0.87	0.37
Ovulatory follicle growth post-dominance[5], mm	4.22	6.46	0.41	< 0.01
Dominant follicle size at luteolysis[6], mm	10.41	13.27	0.80	0.03
Dominant follicle growth post-luteolysis, %	23.64	13.76	4.00	0.11
Proestrus duration[7], h	68.49	36.07	6.16	< 0.01
Ovulatory follicle diameter, mm	13.47	15.40	0.53	0.03
Maximum secondary follicle diameter, mm	8.51	9.80	0.78	0.27
Follicular wavelength, d	9.04	9.59	0.74	0.61
Total ovarian antral follicle count (AFC)				
Day 1 of wave	19.85	17.44	3.18	0.63
Day 2 of wave	17.68	15.53	0.93	0.13
Day 3 of wave	19.14	16.50	1.97	0.37
Average AFC of entire wave	17.56	15.78	1.25	0.34
Corpus luteum volume 7 d post-estrus, cm^3	4.64	4.81	0.74	0.88

[1]Treatment included ad libitum access to corn stalks combined with daily supplementation of either soybean meal (SBM) or corn gluten meal (CGM) to equal 150% of dietary MP requirements.

[2] Greater SEM presented (SBM n = 7; CGM n = 8).

[3] Dominance obtained when largest growing follicle was at least 1mm larger than any other growing follicle and at least 8mm in diameter.

[4] Period between attainment of dominance until ovulation.

[5] Growth of ovulatory follicle between dominance and ovulation.

[6] Luteolysis defined as first day on which circulating progesterone concentrations were < 1 ng/mL.

[7] Period between luteolysis and expression of estrus.

Table 3.4. Effect of source of excess MP supplementation on circulating hormone and PUN concentrations

	Treatment[1]			
Item	SBM	CGM	SEM[2]	*P*-Value
Estradiol-17β at luteolysis, pg/mL	4.6	6.45	1.06	0.24
Peak estradiol-17β, pg/mL	6.62	7.67	0.92	0.44
Change in estradiol-17β	2.02	1.22	0.68	0.46
Estradiol-17β: ovulatory follicle vol, $pg \cdot mL^{-1} \cdot mm^{-1}$	0.49	0.49	0.06	0.99
Progesterone 7 d post estrus, ng/mL	5.70	4.66	0.35	0.07
Progesterone: corpus luteum vol, $ng \cdot mL^{-1} \cdot cm^{3\,-1}$	1.35	1.07	0.16	0.24
PUN at ovulation, mg/dL	8.91	7.62	1.16	0.40

[1]Treatment included ad libitum access to corn stalks combined with daily supplementation of either soybean meal (SBM) or corn gluten meal (CGM) to equal 150% of dietary MP requirements.

[2] SEM (estradiol and progesterone : SBM n = 7, CGM n = 8; PUN: SBM n = 9, CGM n = 9).

Table 3.5 Effects of excess MP supplementation on plasma AA concentrations.

	Treatment[1]			
Item	SBM	CGM	SEM[2]	*P*-Value
AA, umol/L[3]				
Total AA	1,573	1,682	83.75	0.37
Essential AA	791.07	829.53	56.23	0.64
Nonessential AA	782.24	852.9	41.95	0.25
Glycogenic AA	939.10	1007	48.26	0.33
Ketogenic AA	207.33	263.02	20.06	0.07
Branched-chain AA	660.75	733.7	39.39	0.21
Essential AA				
Arginine	85.68	73.49	7.39	0.26
Histidine	52.89	58.14	3.55	0.31
Isoleucine	82.66	84.27	5.84	0.85
Leucine	123.79	199.13	17.47	< 0.01
Lysine	83.54	63.88	8.83	0.14
Methionine	18.30	22.48	1.45	0.06
Phenylalanine	48.79	67.00	4.54	0.01
Threonine	56.41	44.15	6.43	0.20
Tryptophan	32.87	23.69	3.92	0.12
Valine	206.14	193.29	14.09	0.53
% of Total AA[3]				
Essential AA	49.73	49.34	1.75	0.88
Nonessential AA	50.27	50.66	1.75	0.88
Glycogenic AA	60.21	59.81	1.61	0.86
Ketogenic AA	12.83	15.69	0.74	< 0.01
Branched-chain AA	41.92	43.68	1.01	0.23
Essential				
% Arginine	5.44	4.34	0.33	0.03
% Histidine	3.34	3.47	0.14	0.50
% Isoleucine	5.21	5.03	0.23	0.59
% Leucine	7.59	11.94	0.80	< 0.01
% Lysine	5.24	3.75	0.40	0.02
% Methionine	1.15	1.33	0.05	0.03
% Phenylalanine	3.10	4.02	0.25	0.02
% Threonine	3.49	2.58	0.30	0.05
% Tryptophan	2.09	1.39	0.21	0.03
% Valine	13.08	11.47	0.54	0.05
Nonessential				
% Alanine	9.99	10.36	0.58	0.66
% Asparagine	1.68	1.90	0.08	0.05
% Aspartate	0.33	0.37	0.02	0.19
% Glutamate	4.02	4.01	0.22	0.99
% Glutamine	16.04	15.23	0.62	0.37
% Glycine	11.55	10.64	0.96	0.51
% Proline	3.33	4.53	0.24	< 0.01

Table 3.5 continued.				
% Serine	3.35	3.62	0.13	0.17

[1]Treatment included ad libitum access to corn stalks combined with daily supplementation of either soybean meal (SBM) or corn gluten meal (CGM) to equal 150% of dietary MP requirements.
[2] SEM: SBM n = 9, CGM n = 9.
[3]Cysteine and Tyrosine are not presented as these AA were not present at detectable concentrations in samples.

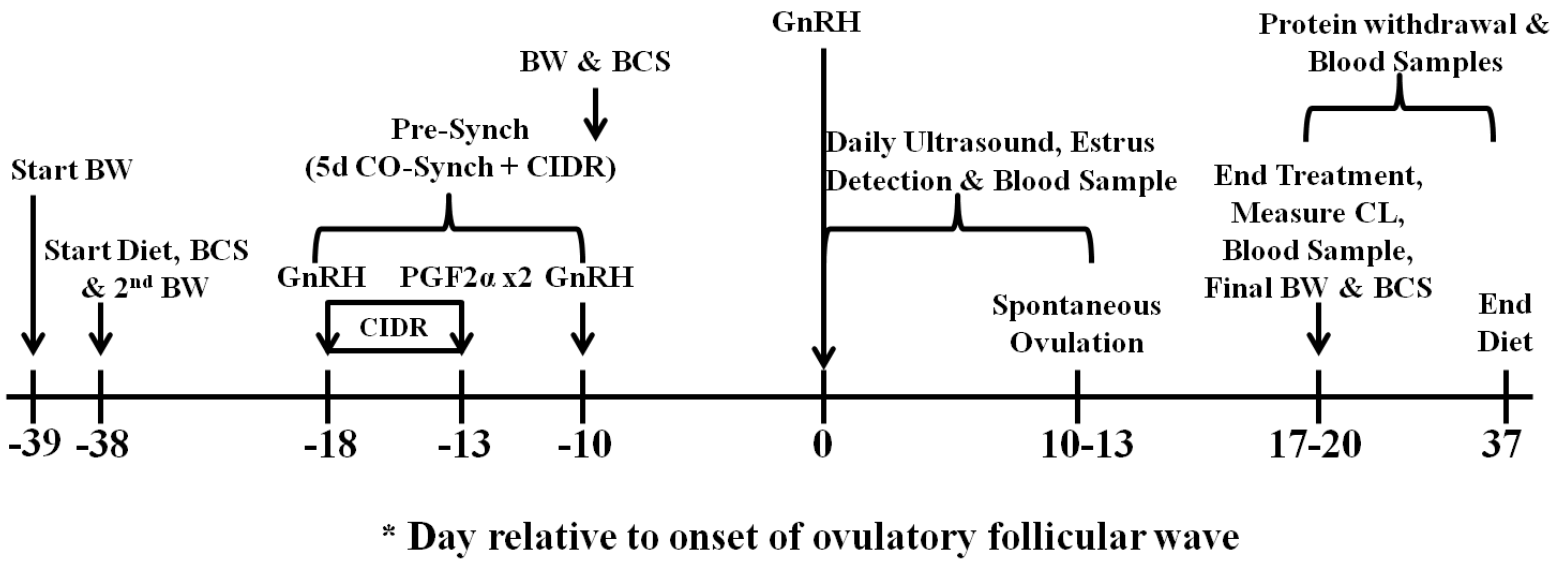

Figure 3.1. Experimental design for treatments (CGM = corn gluten meal supplement, high RUP source; SBM= soybean meal supplement, low RUP source) outlining treatments and data collection relative to onset of ovulatory follicular wave. Protein was supplemented from d -38 to d 19 and corn stalks were supplemented from d -38 to d 37.

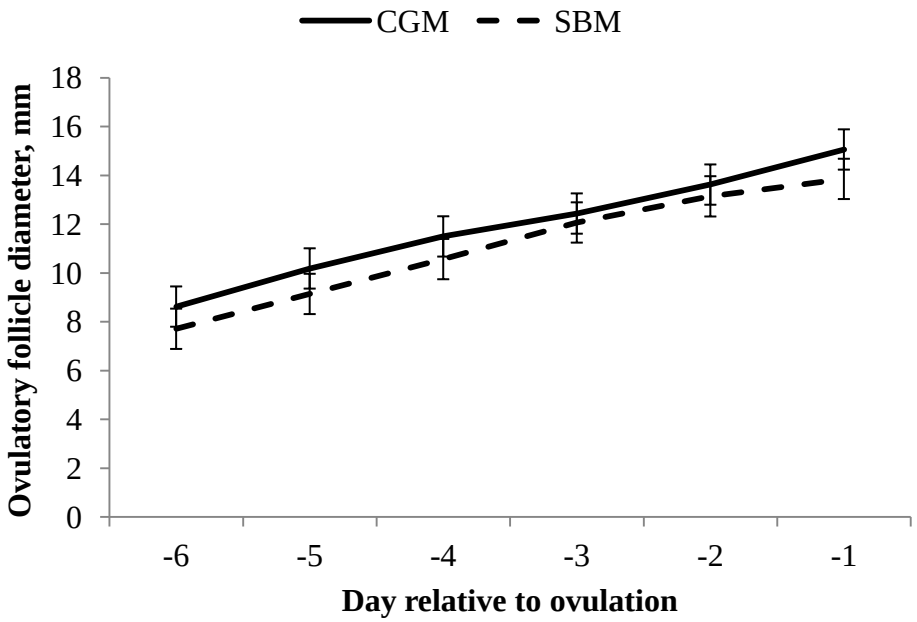

Figure 3.2. Effect of treatment (CGM = corn gluten meal supplement, high RUP source; SBM= soybean meal supplement, low RUP source) on ovulatory dominant follicle growth 6 d pre-ovulation. An effect of day ($P < 0.001$) was observed. *P*-values for treatment and treatment × day interaction were 0.42 and 0.69, respectively.

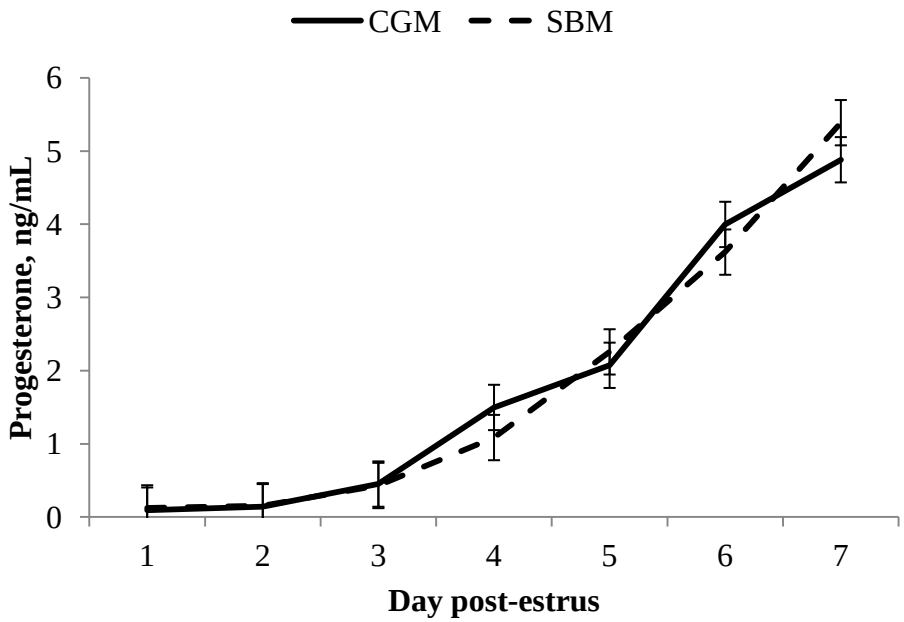

Figure 3.3. Effect of treatment (CGM = corn gluten meal supplement, high RUP source; SBM= soybean meal supplement, low RUP source) on circulating progesterone concentrations 7 d post-estrus. An effect of day ($P < 0.001$) was observed. *P*-values for treatment and treatment × day interaction were 0.97 and 0.19, respectively.

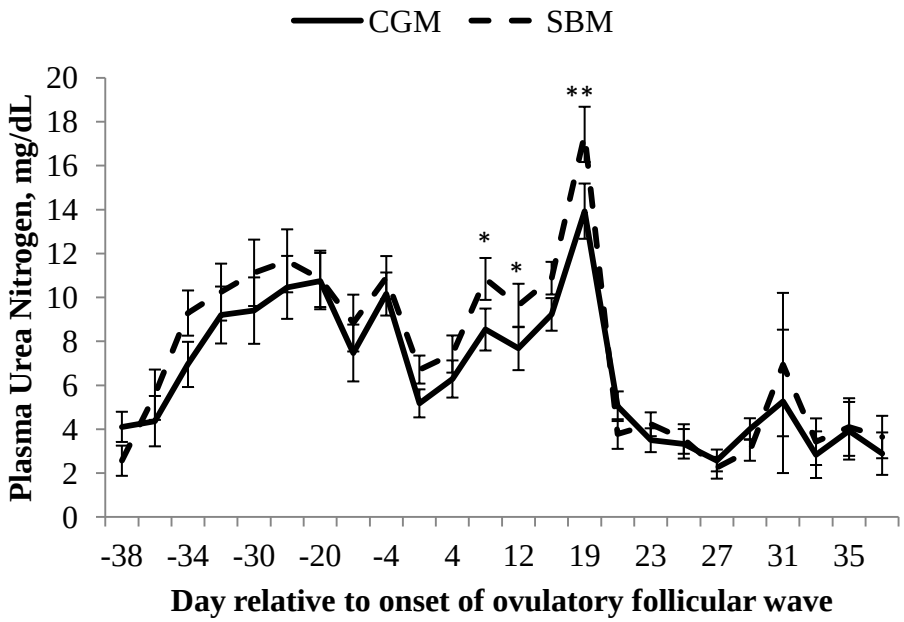

Figure 3.4. Effect of treatment (CGM = corn gluten meal supplement, moderately abundant RUP source; SBM = soybean meal supplement, low RUP source) on plasma urea nitrogen concentrations. Protein supplements were initiated on d -38 and continued until d 19, and thereafter libitum corn stalks were offered to observe PUN return to baseline. There was a treatment × day interaction ($P = 0.02$) observed. *P*-values for treatment and day were 0.40 and < 0.001, respectively. Days on which PUN was different between treatments ($P \leq 0.05$ and $P < 0.10$) are denoted by ** and *, respectively.

CHAPTER 4.

EFFECTS OF SUPPLEMENTING EXCESS AMOUNTS OF METABOLIZABLE PROTEIN FROM A MODERATELY ABUNDANT RUMEN UNDEGRADABLE SOURCE ON OVARIAN FUNCTION AND CIRCULATING AMINO ACID CONCENTRATIONS OF BEEF COWS CONSUMING LOW QUALITY FORAGE

4.1 ABSTRACT

The objective of this experiment was to determine the effects of excess MP supplementation from a moderately abundant RUP source (corn gluten: 62% RUP) on ovarian function and circulating AA concentrations in beef cows consuming low quality forage. Non-pregnant, non-lactating multiparous beef cows (n = 16) were allocated by age, BW and BCS to 1 of 2 isocaloric supplements designed to maintain BW for 60-d. Each day, cows had ad libitum access to processed corn stalks and were individually offered a corn gluten meal-based supplement at 125% (MP125) or 150% (MP150) of NRC MP requirements. After a 20 d supplement adaptation period, cows were synchronized for ovulation using the 5-d CO-Synch + CIDR protocol. Ten days after synchronization completion, 100 µl of GnRH was administered to reset follicular growth. Starting at GnRH administration and daily thereafter, transrectal ultrasonography was performed to diagram ovarian follicular waves, and blood samples were collected for hormone, metabolite, and AA analyses. Seven days after observation of estrus, corpus luteum (CL) size was determined via ultrasound and supplementation was ended. Data

were analyzed using the MIXED procedures of SAS. As designed, no differences ($P \geq 0.21$) in BW and BCS existed at the beginning or end of the study; however, plasma urea N at ovulation was greater ($P = 0.04$) in MP150 cows. Preovulatory ovarian follicle characteristics including size at dominance, duration of dominance, post-dominance growth, size at spontaneous luteolysis, post-luteolysis growth, length of proestrus, and wavelength were not different ($P \geq 0.11$) between treatments. However, ovulatory follicles were larger ($P = 0.04$) and average antral follicle count was greater ($P = 0.01$) in MP150 than MP125 treatment. Estradiol concentrations and ratio of estradiol to ovulatory follicle volume were not different due to treatment ($P \geq 0.25$). While CL volume 7 d post-estrus was greater ($P < 0.01$) in MP150 than MP125 cows, circulating progesterone 7 d post-estrus and ratio of progesterone to CL volume were not different ($P \geq 0.21$). Total AA were not different ($P \geq 0.76$) at study initiation or completion; however, as a percent of total AA, branched-chain AA at ovulation were greater ($P = 0.02$) in MP150 treatment. In conclusion, supplementation of CP at 150% of NRC MP requirements from a moderately undegradable protein source may enhance growth of the ovulatory follicle and subsequent CL compared to MP supplementation at 125% of NRC MP requirements.

4.2 INTRODUCTION

Protein supplementation with ethanol coproducts has become a common practice for beef cattle producers in the Midwest, especially as the corn ethanol industry continues to expand. Ethanol coproducts are a condensed source of protein and energy, which when paired with low quality forage, are capable of meeting nutrient demands of beef cows (Geppert & Gunn, 2014). However, with increasing oil extraction occurring during the

ethanol process, ethanol coproducts are becoming more concentrated in protein and are replacing traditional coproducts (U.S. Grains Council, 2012). When these higher protein coproducts are supplemented as a primary energy source, total dietary CP may exceed 150% of NRC requirements. This is of particular concern as excess dietary CP has been associated with reduced fertility in dairy cattle (Ferguson et al., 1993; Butler et al., 1996). However, more recent studies have demonstrated that supplementation of excess dietary protein (DDGS) may be beneficial to reproductive performance of primiparous beef heifers (Gunn et al., 2014b) and pregnancy rates of beef cows (Gunn et al., 2014a).

More specifically, when protein from a moderately rumen undegradable source (62% RUP) was offered to equal approximately 150% of NRC MP requirements ovulatory follicle diameter of non-lactating beef cows were enhanced (Gunn et al., 2014c; Geppert et al., 2015). However, more research is warranted to determine if amount of excess MP from a moderately RUP feedstuff differentially effects ovarian function of beef cows. Therefore, the objective of this experiment was to determine if the amount of excess dietary protein (125% MP vs. 150% MP) from a moderately rumen undegradable feedstuff affected ovarian function of beef cows consuming low quality forages. We hypothesized that increasing the amount of excess MP supplemented from a moderately rumen undegradable source would enhance ovarian parameters of Ovulatory follicle diameter, corpus luteum development and hormone profiles around the time of ovulation, compared to excess MP at a decreased supplementation rate.

4.3 MATERIALS AND METHODS

4.3.1 ***Animals and diets.*** All protocols and procedures used were approved by the Iowa State University Institutional Animal Care and Use Committee. The project was conducted at Zumwalt Station Research Unit in Ames, Iowa from May 2014 to July 2014. In order to evaluate the effects of excess amounts of MP from a moderately undegradable protein source on ovarian function around ovulation, 16 non-pregnant, non-lactating, Angus and Angus-Simmental multiparous beef cows were used. Cows were stratified by age (6.36 ± 2 yr), BCS (4.93 ± 0.34 [1 = emaciated, 9 = obese; Wagner et al., 1988]) and BW (552 kg ± 36 kg) and allotted to 1 of 2 isocaloric dietary treatments differing in amount of excess MP inclusion (Table 4.1). All cows were offered ad libitum access to processed corn stalks (6% CP and 80% NDF) and individually supplemented once daily with a moderately undegradable protein supplement (corn gluten meal: 62% RUP) for 60-d (Figure 4.1). Supplements were formulated to provide excess MP at either: 1) 125% (**MP125**) or 2) 150% (**MP150**) of MP requirements (NRC, 2000). Diet formulations were designed from feedstuffs analyzed at the beginning of the trial (Dairyland Laboratories Inc., Arcadia, WI) to maintain similar cow BW and BCS throughout the study. Although diets were not isonitrogenous, balancing diets using MP ensured that the RDP requirements were met or exceeded for cows in both treatments.

Daily individual supplementation took place at 0800 h inside a facility where 10 side-by-side stanchions were located. All cows from 1 treatment (n = 8) were restrained in stanchions and allowed to consume supplement at the same time. Cows were allowed adequate time to consume supplement while daily samples and measurements were being

recorded. After consumption of supplement and data were collected, cows were released from stanchions and the next treatment group was brought in to the facility.

4.3.2 ***Performance characterization.*** Initial BCS was assessed on the day prior to trial initiation and was recorded as the average of scores from 2 trained investigators. In addition, initial BW was recorded as the average BW measured on the day prior to and the first day of the trial, before supplement delivery. Additional BCS and BW were taken once monthly, with BW being averaged on 2 consecutive days prior to treatment delivery, and cows had restricted access to corn stalks for 12 h prior to BW collection to minimize variation in gut fill. Final BCS was taken on the last day of supplementation, and final BW was taken prior to supplementation on the day prior to and the last day of the study.

4.3.3 ***Experimental design.***

4.3 3.1 Pre-synchronization. Treatment experimental design is outlined in Figure 1. Twenty days after initiating dietary treatments, cows were synchronized with the 5-day CO-synch + CIDR protocol. At synchronization initiation, cows were administered 100 µg of GnRH (Cystorelin; Merial LLC, Duluth, GA) and received a **CIDR** (EAZI-BREED, Zoetis Animal Health, New York, NY). Five days after GnRH administration and CIDR insert, the CIDR was removed and cows were administered 25 mg $PGF_{2\alpha}$ (Lutalyse; Zoetis Animal Health, New York, NY), with a second 25-mg injection of $PGF_{2\alpha}$ administered 8 h after the first. Also at the time of CIDR removal, an Estrotect™ heat detection aid (Rockway Inc., Spring Valley, WI) was placed on the tailhead of each cow to assist in estrus detection, which was performed by trained personnel twice daily for 30 min each at 0630 h and 1900 h. Seventy-two hours after CIDR removal and initial

$PGF_{2\alpha}$ injection, all cows received 100 µl GnRH and heat detection aid activity was recorded and removed.

4.3.3.2 ***Ovarian follicular wave characterization.*** Ten days after synchronization protocol completion, 100 µg of GnRH was administered to initiate growth of a new follicular wave. Beginning at GnRH administration and daily thereafter at 0800 h, cows were subject to transrectal ultrasonography (Ibex™ Portable Ultrasound, variable MHz linear array transducer, E.I. Medical Imaging, Loveland, CO) for complete characterization of a single follicular wave. Location and size of all antral follicles ≥ 3 mm in diameter were recorded each day by drawing sketches of each ovary. Transrectal ultrasound examinations were performed by the same investigator for the duration of the ultrasound period and estrus detection was performed twice daily by trained personnel at 0630 h and 1700 h with the aid of heat detector patches. All follicle measurements were made using the internal caliper function of the ultrasound, with final reported follicle diameters being the average of the greatest two cross-sectional perpendicular measurements of the follicle.

Day of dominance and size at dominance were categorized on the day of which the largest growing follicle was at least 8 mm in diameter and at least 1 mm larger in diameter than any other growing follicle of the same wave. The dominant follicle was documented as the largest growing follicle in the wave of interest, and the secondary follicle was the second largest growing follicle in the same wave. Duration of dominance was retrospectively determined as the number of days from attainment of dominance until day of ovulation. Daily ovarian ultrasound ended upon successful ovulation as confirmed

by disappearance of the ovulatory follicle from the wave of interest, preceded by visual display of estrus.

When estrus and subsequent ovulation were both confirmed, ultrasound measurements were halted until 7 d post-estrus, where ultrasound was performed once again to measure the total CL volume. Volume of the CL was determined using the internal caliper function of the ultrasound and formula for a rotary ellipsoid (V= 4/3 Π Iab^2, where *a*= longitudinal axis and *b*= transverse axis), minus the volume of a lacunae (calculated similarly) if present. After final CL measurement, dietary treatments ended.

Day of spontaneous luteolysis was retrospectively determined using the first day on which progesterone concentrations were ≤ 1 ng/mL via RIA. This also allowed ovulatory follicle size at spontaneous luteolysis and growth from luteolysis to ovulation to be determined. Length of proestrus was also retrospectively determined as the hr between spontaneous luteolysis and expression of estrus. Wave emergence was tracked by tracing the dominant follicle back to a cohort of follicles ≤ 4 mm in diameter, and wavelength was calculated as total days from wave emergence to ovulation. Antral follicle counts were totaled daily and all days were averaged to calculate average AFC of the entire wave.

4.3.4 ***Plasma analyses.*** During the 20-d dietary adaptation period, coccygeal blood samples were collected twice weekly, and plasma was stored for later analysis of PUN concentration. During the ultrasound period until final CL measurement 7 d post-estrus, coccygeal blood samples were taken daily for plasma analysis of PUN, estradiol-17β and progesterone concentrations. At collection, approximately 4 mL of blood was collected via coccygeal venipuncture in a 6 mL EDTA vacutainer (10.8 mg of EDTA;

BD Vacutainer™; Becton, Dickinson and Co., Franklin Lakes, NJ) and immediately placed on ice. Samples were centrifuged at 1,750 x *g* for 25 minutes at 4° C, and plasma was recovered and transferred into 5 mL polystyrene tubes and frozen at -20°C until respective analyses were conducted.

Plasma samples collected during the ultrasound period were analyzed for circulating progesterone concentrations to determine day of spontaneous luteolysis and subsequent CL progesterone secretion. Concentrations were determined using a commercially available RIA kit (Coat-A-Count, Siemens Healthcare Diagnostics Inc., Los Angeles, CA). Across 3 assays, the average intra-assay CV was 1.2%, with the inter-assay CV from pooled samples containing 0.59 ng/mL and 5.82 ng/mL, was 9.8 % and 6.7% respectively. Average sensitivity across the assays was 0.14 ng/mL (95% confidence interval). Plasma samples collected from the day of spontaneous luteolysis until ovulation were analyzed for circulating concentrations of estradiol-17β via RIA at South Dakota State University following methods described by Perry and Perry (2008). Across 3 assays, the average intra-assay CV was 4.5 % and the average inter-assay CV from a pooled serum sample containing 7.9 pg/mL was 8%. The average sensitivity across 3 assays was 0.49 pg/mL (95% sensitivity).

Plasma samples were analyzed for PUN using a commercially available assay (Urea Nitrogen Procedure No. 0580, Stanbio Laboratory, Boerne, TX). Samples and standards were prepared, loaded into a 96 well plate and read on an Eon Microplate Spectrophotometer at 520 nm (BioTek Instruments, Inc., Winooski, VT). Across 8 assays, the average intra-assay CV was 5.3%, with an inter-assay CV from pooled serum containing 7.49 mg/dL of urea N was 10.9 %. Preprandial blood samples taken at

treatment initiation and around time of estrus (d 15) were analyzed for circulating AA concentrations at North Dakota State University via methods of Ultra Performance Liquid Chromatography (Lemley et al., 2013).

4.3.5 ***Statistical analysis.*** Data were analyzed using the MIXED procedure of SAS 9.3 (SAS Inst. Inc., Cary, NC), with the main effect of treatment and experimental unit of animal. For ovarian follicular wave characteristics, hormone and metabolite analyses, the covariates of age and individual change in BW were included in the model as covariates. The MIXED procedures of SAS for REPEATED measures were utilized to analyze ovulatory follicle growth, progesterone and PUN concentrations. The covariance structures compound symmetry, heterogeneous compound symmetry, autoregressive order one, unstructured and ante-dependence were compared. The covariance structure resulting in the smallest Bayesian information criterion was used for the final analyses. The effects of treatment and day, as well as the interaction of treatment and day were analyzed in the model. Length of time from visual display of estrus to final CL measurement was also run as a covariate in the repeated analyses; however, it was removed from the model due to insignificance. The simple effects within day were determined using the SLICE function of SAS.

For all analyses, significance was noted at a P-value ≤ 0.05, with tendencies identified at P-value of > 0.05 and ≤ 0.10. It should be noted that 2 cows from the MP125 treatment and 3 cows from the MP150 treatment failed to reach progesterone concentrations > 1 ng/mL after ovulation; therefore, all data from those 5 cows were removed from the study.

4.4 RESULTS

4.4.1 ***Performance characteristics.*** Performance characteristics of BW and BCS are presented in Table 4.2. As designed, BW and BCS were similar ($P \geq 0.74$) between treatments at initiation of the study. Furthermore, with use of isocaloric dietary treatments, final BW and BCS were not different ($P \geq 0.73$) at the conclusion of the trial.

4.4.2 ***Ovulatory follicle wave characteristics.*** Ovulatory follicle wave and CL characteristics are presented in Table 4.3. The preovulatory follicle parameters of wavelength, size at dominance, duration of dominance, and growth post-dominance as well as size at spontaneous luteolysis, growth post-luteolysis and duration of proestrus were not different ($P \geq 0.11$) among treatments. However, cows from the MP150 treatment had greater maximum ovulatory follicle diameter ($P = 0.04$) and average AFC ($P = 0.01$) compared to the MP125 treatment. In addition, there was a significant ($P < 0.02$) treatment × day interaction for ovulatory follicle size post-emergance, as cows from MP150 treatment had larger ($P \leq 0.05$) ovulatory follicles on d 10 and 11 post-emergence than MP125 cows (Fig. 4.2). Lastly, CL volume 7 d post-estrus was greater ($P < 0.01$) in MP150 than MP125 treatment cows.

4.4.3 ***Plasma analyses.***

Hormones. Circulating estradiol concentrations at luteolysis, as well as peak concentration prior to onset of estrus were not different between dietary treatments ($P \geq 0.51$; Table 4.4). Furthermore, when the ratio of estradiol to ovulatory follicle volume was accessed, no difference between treatments existed ($P = 0.98$). In addition, no differences in circulating progesterone or the ratio of progesterone to CL volume ($P \geq 0.21$) were seen due to dietary treatment. Although not significantly different ($P > 0.10$),

circulating progesterone concentrations were numerically greater in MP150 treatment at 7 d post-estrus (Fig. 4.3).

Plasma urea nitrogen. Initial PUN concentrations were not different ($P = 0.17$; Table 4.4) between treatments. However, there was a treatment × day interaction detected ($P < 0.01$; Fig. 4.4), as PUN concentrations were greater ($P < 0.05$) in MP150 compared to MP125 cows after onset of ovulatory follicular wave.

Amino acid. Amino Acid concentrations prior to treatment initiation and at the time of ovulation are located in Table 4.5 and Table 4.6, respectively. At trial initiation before protein supplementation, total AA, as well as total essential AA and nonessential AA were similar ($P \geq 0.81$) between dietary treatments. At ovulation, plasma samples showed no difference ($P \geq 0.77$) in total circulating AA or total essential and nonessential AA concentrations between treatments. However, circulating total phenylalanine tended to be greater ($P = 0.09$) in MP150 than MP125 treatment. In addition, when expressed as a percent of total AA, branched-chain AA (**BCAA**) were greater ($P = 0.02$) in MP150 compared to MP125.

4.5 DISCUSSION

4.5.1 ***General.*** The objective of this study was to determine if MP fed at 125% of MP requirements from a moderately undegradable source would have similar effects on beef cow ovarian function as supplementation at 150%. Diets containing increased CP have historically resulted in elevated circulation of urea N. This may be of concern as PUN concentrations above 19 mg/dL have been associated with suppressed fertility in dairy cows (Ferguson and Chalupa, 1989; Elrod and Butler, 1993; Butler et al., 1996).

However, feeding protein at 150% of NRC requirements has not been shown to result in PUN exceeding 19 mg/dL in beef cattle (Rusche et al., 1993; Gunn et al., 2014b). Furthermore, these studies showed no suppression of reproductive parameters or fertility.

Few studies have explored the relationship between excess rumen undegradable protein, PUN, and reproductive parameters in beef cattle. However, it was recently elucidated that excess CP from a more concentrated rumen undegradable feedstuff (corn gluten meal) enhanced ovulatory follicle parameters of non-lactating females compared to 150% MP from a more degradable source (soybean meal) (Geppert et al., 2015). Gunn et al. (2014c) also reported enhanced ovulatory follicle diameter, antral follicle counts and estradiol concentrations from cows fed 150% MP when compared to 100% MP.

In the present study, supplementation of RUP at 150% or requirements resulted in greater PUN circulation than 125%, which was expected based on previous research by Sletmoen-Olson et al. (2000), as a linear increase in PUN was observed with greater RUP inclusion. Nonetheless, while PUN concentrations were elevated compared to baseline in both treatment groups after supplementation, circulating PUN was still relatively low compared to concentrations known to be associated with decreased reproductive performance.

4.5.2 *Ovulatory follicle wave characteristics*

Previous studies reported that excess CP supplementation yielded larger ovulatory follicles in lactating cows (Lents et al., 2008) and first-calf heifers (Gunn et al., 2014b). In addition, recent research in our lab (Gunn et al., 2014c; Geppert et al., 2015), in agreement with the current study, showed that excess CP sourced from corn gluten meal, a moderately abundant RUP source, supplemented at 150% of NRC requirements

enhanced ovulatory follicle size compared to diets containing 100% of MP requirements from gluten meal and 150% of MP requirements from soybean meal (64% RDP) in non-lactating, non-pregnant beef cows, respectively. Ovulatory follicle size has been associated with enhanced pregnancy success, as spontaneous ovulation of large follicles in beef heifers were more likely to result in a pregnancy than females ovulating smaller follicles (Perry et al., 2007). However, pregnancy success was not correlated with follicle size when cows underwent spontaneous ovulation (Perry et al., 2005). The disconnect between pregnancy success, spontaneous ovulation and follicle size in beef heifers vs. cows could be due to differences in age and stage of development of the females (Santos et al., 2001). Thus, beef cows having lesser nutrient demands than developing heifers may be able to utilize excess MP to increase ovarian follicular development and pregnancy support, regardless of induced or spontaneous ovulation.

In addition, greater ovulatory follicle diameter could potentially be due to the increased proportion of bypass protein in the MP150 vs. MP125, which may in turn increase circulating insulin concentrations (Sletmoen-Olson et al., 2000; Schroeder and Bauer, 2005). Greater circulating insulin has been found to be associated with enhanced dominant follicle size (Ciccioli et al., 2003), potentially by working with GH and insulin like growth factor-1 (IGF-1) to control follicular growth (Diskin et al., 2003; Silva et al., 2009). However, we did not measure insulin, GH or IGF-1 concentrations in the present study so the greater follicular growth could be due to a variety of the aforementioned mechanisms.

It has been previously demonstrated, as is here, that excess dietary RUP supplementation yields greater average antral follicle counts (Gunn et al., 2014c). Greater

AFC may result in improved fertility potentially due to improved oocyte competence compared to lower AFC cows (Ireland et al., 2011). However, the exact mechanism of how AFC is mediated is widely unknown. Cushman et al. (2014) suggested that AFC may be predetermined prior to birth, but may also be influenced through developmental programming. Yet, to our knowledge this study and research by Gunn et al. (2014c) are the first reports suggesting that diet may alter AFC. While initial AFC measurements were not taken in either of the previously mentioned studies, the likelihood of two similar experiments seeing AFC differences is intriguing. Although only different by a small margin, AFC in MP150 cows was shifted from low to intermediate AFC as outlined by Ireland et al. (2011), potentially improving fertility in females from the MP150 treatment. However, as the primordial germ cell population is established prior to birth, enhancing follicle recruitment or AFC may shorten the reproductive lifespan of a female by depleting the follicular reserve more quickly. Yet, as AFC begins decreasing in bovine females after 5 yrs of age (Cushman et al., 2009), perhaps excess protein fed at 150% MP requirements may be beneficial to follicular recruitment in females past peak AFC production.

Cows induced to ovulate large follicles have been reported to develop larger CL compared to their smaller counterparts (Vasconcelos et al., 2001). As the CL develops from the granulosa and theca cells of the ovulatory follicle, thus larger follicles tend to have more luteal cells and greater estradiol exposure which influence the developing luteal tissue after ovulation (Atkins et al., 2013). Smaller CL development in MP125 cows was expected after ovulating smaller follicles. Small CL formation could also be due to inadequate estradiol exposure which could have led to ovulation of an unhealthy

follicle, and in turn may impair vasculature of the subsequent CL disrupting its development (Tamanini and De Ambrogi, 2004). However, normal development of the CL is also dependent on blood flow (Acosta and Miyamoto, 2004) which is also associated with AA (arginine) through nitric oxide mechanisms (Vonnahme et al., 2005). Thus, differences in CL volume could also be due to differences in AA between treatments potentially mediating blood flow and subsequent CL function.

4.5.3 *Hormones and AA*

Given differing ovulatory follicle sizes, it was surprising that we did not observe a difference in estradiol concentrations due to treatment. One would expect greater circulating estradiol concentrations at ovulation of the larger follicles (Vasconcelos et al., 2001; Perry et al., 2005; Busch et al., 2008); however, similar proestrus intervals between treatments may have mitigated differences in estradiol production (Bridges et al., 2010). In addition, as size of the ovulatory follicle has been associated with subsequent CL volume (Vasconcelos et al., 2001), a correlation between CL volume and circulating progesterone has also been observed in beef heifers (Eborn et al., 2013) and cows (Echternkamp et al., 2009). However, regardless of follicle and CL size in the present study, no difference in subsequent circulating progesterone existed, potentially due to ample hormone priming (McNatty et al., 1981) allowing luteal formation in both treatments (Busch et al., 2008).

Regardless of insignificant differences in estradiol and progesterone concentrations, the decreased production of essential hormones in both treatments is slightly concerning as certain concentrations must be present in order to successfully maintain normal estrous cycles and attain pregnancy. As a positive correlation between

estradiol concentration at estrus and subsequent progesterone concentration at 7 d post-estrus exists (Jinks et al., 2012), it was not surprising that both hormone concentrations followed similar patterns. Other authors have observed no alterations to hormone production after excess RUP supplementation (Gunn et al., 2014c; Geppert et al., 2015); therefore, suppressed estradiol and progesterone concentrations regardless of treatment in this study may have been due to seasonal effects of heat stress as reviewed by Rensis and Scaramuzzi (2003).

Recent research in our lab has demonstrated that excess CP is capable of shifting circulating AA concentrations in beef cows consuming low quality forage (Geppert et al., 2015). In particular the aforementioned study noted an increase in total leucine and leucine as a percent of total circulating AA. As leucine, isoleucine, and valine comprise BCAA, the links between these two studies are intriguing as little is known about the influence of circulating AA on ovarian function. However, greater BCAA in circulation could be contributing to enhanced ovarian parameters through enhancing ovarian tissue synthesis with the use of the mammalian target of rapamycin (mTOR) activation pathway (Greiwe et al., 2001; Herman et al., 2010), as observed in rat granulosa cells (Yu et al., 2011) and bovine luteal tissue (Zhang et al., 2011). However, as this is one of this first reports on the relationship between AA and ovarian function in beef cows, further investigation is warranted.

4.5.4 *Conclusion.*

In summary, feeding excess RUP at 125% and 150% MP resulted in differential effects on ovarian reproductive parameters. While circulating hormone concentrations were similar between treatments, ovulatory follicle and CL parameters were enhanced by

excess RUP at 150% of MP requirements compared to 125%. Furthermore, cows fed 150% MP had greater BCAA circulation, which appears to have a potential role in the increased growth of ovulatory follicles and CL development. Therefore, supplementation of CP at 150% of NRC MP requirements from a moderately undegradable protein source appears to enhance growth of the ovulatory follicle and the subsequent CL compared to MP supplementation at 125% of requirements. While effects of excess RUP at the ovarian level were accessed here, a continuation of this study should be conducted to further investigate the impacts of excess MP from a moderately rumen undegradable source on overall fertility.

Table 4.1. Supplement provided to cows consuming ad libitum corn stalks[2]

	Treatment[1]	
Item	MP125	MP150
Dry matter intake, kg/d		
Corn silage	0.25	0.25
Corn gluten meal[3]	0.31	0.67
Cracked corn	0.32	--
Mineral	0.11	0.11
Calculated nutrient intake of supplement		
CP, kg/d	0.26	0.48
RUP, kg/d	0.37	0.41
NE_m, Mcal/kg	1.80	1.83
NE_g, Mcal/kg	1.22	1.23

[1]Treatment included ad libitum access to corn stalks, followed by daily supplementation of MP at 125% (MP125) or 150% (MP150) of NRC MP requirements for non-pregnant, non-lactating multiparous beef cows.
[2] Corn stalk nutrient analysis (% DM basis): 51% TDN; 6% CP; 50% ADF; 80% NDF.
[3] Nutrient analysis (% DM basis): 47% CP; 62% RUP; 2.4% Fat.

Table 4.2. Effects of excess amounts of MP supplementation on BW and BCS performance

	Treatment[1]			
Item	MP125	MP150	SEM	*P*-Value
BW, kg				
Initial	548.81	556.74	16.96	0.74
Final	560.56	551.16	19.57	0.73
BCS[2]				
Initial	4.95	4.90	0.16	0.84
Final	4.84	4.83	0.11	0.99

[1]Treatment included ad libitum access to corn stalks, followed by daily supplementation of MP at 125% (MP125) or 150% (MP150) of NRC MP requirements for non-pregnant, non-lactating multiparous beef cows.
[2]BCS on scale of 1 to 9 (1 = emaciated, 9 = obese; Wagner et al, 1988).

Table 4.3. Effects of excess amounts of MP supplementation on ovulatory follicle wave and corpus luteum characteristics

	Treatment[1]			
Item	MP125	MP150	SEM[2]	*P* -Value
Ovulatory follicle size at dominance[3], mm	8.31	8.53	0.32	0.65
Dominance duration[4], d	6.65	6.62	1.27	0.99
Ovulatory follicle growth post-dominance[5], mm	4.37	6.81	0.94	0.11
Dominant follicle size at luteolysis[6], mm	11.58	13.52	1.38	0.40
Dominant follicle growth post-luteolysis, mm	2.37	1.51	0.77	0.54
Proestrus duration[7], h	33.82	45.38	10.43	0.50
Ovulatory follicle diameter, mm	12.60	15.28	0.73	0.04
Maximum secondary follicle diameter, mm	7.90	7.92	0.69	0.99
Follicular wavelength, d	10.73	10.73	0.87	0.99
Total ovarian antral follicle count (AFC)				
Day 1 of wave	19.28	17.26	1.57	0.40
Day 2 of wave	15.43	18.28	1.73	0.29
Day 3 of wave	16.99	18.42	2.10	0.65
Average AFC of entire wave	13.72	16.68	0.62	0.01
Corpus luteum volume 7 d post-estrus, cm^3	1.17	6.08	0.56	< 0.01

[1]Treatment included ad libitum access to corn stalks, followed by daily supplementation of MP at 125% (MP125) or 150% (MP150) of NRC MP requirements for non-pregnant, non-lactating multiparous beef cows.

[2] Greater SEM presented (MP125: n = 6; MP150: n= 5).

[3] Dominance obtained when largest growing follicle was at least 1mm larger than any other growing follicle and at least 8mm in diameter.

[4] Period between attainment of dominance until ovulation.

[5] Growth of ovulatory follicle between dominance and ovulation.

[6] Luteolysis defined as first day on which circulating progesterone concentrations were < 1 ng/mL.

[7] Period between luteolysis and expression of estrus.

Table 4.4. Effects of excess amounts of MP supplementation on estradiol, progesterone and plasma urea nitrogen concentrations

	Treatment[1]			
Item	MP125	MP150	SEM[2]	*P*-Value
Estradiol-17β at luteolysis, pg/mL	4.34	5.02	1.33	0.74
Peak estradiol-17β, pg/mL	5.86	6.97	1.10	0.51
Change in estradiol-17β, pg/mL	1.75	2.02	0.58	0.76
Estradiol-17β: ovulatory follicle vol, $pg \cdot mL \cdot mm^{-1}$	0.46	0.46	0.08	0.98
Progesterone 7-d post-estrus, ng/mL	2.23	3.39	0.63	0.25
Progesterone: corpus luteum vol, $ng \cdot mL^{-1} \cdot cm^{3\,-1}$	1.70	0.36	0.65	0.21
Initial PUN, mg/dL	2.20	2.86	0.32	0.17
PUN at ovulation, mg/dL	5.94	8.59	0.82	0.04

[1]Treatment included ad libitum access to corn stalks, followed by daily supplementation of MP at 125% (MP125) or 150% (MP150) of NRC MP requirements for non-pregnant, non-lactating multiparous beef cows.

[2] Greater SEM presented (MP125: n = 6; MP150: n = 5).

Table 4.5. Initial circulating AA concentrations of multiparous beef cows before supplementation with excess amounts of MP

	Treatment[1]			
AA, umol/L	MP125	MP150	SEM[2]	*P*-value
Total AA	1532	1557	86.68	0.83
Essential AA	584.94	603.37	56.19	0.81
Nonessential AA	946.91	953.8	41.46	0.91
Glycogenic AA	1009	1019	44.54	0.88
Ketogenic AA	161.44	173.58	18.9	0.65
Branched-chain AA	554.46	554.29	50.09	0.99
Essential AA				
Histidine	36.68	37.20	3.21	0.91
Arginine	40.83	40.67	5.88	0.98
Threonine	46.13	47.16	4.71	0.88
Lysine	75.56	83.03	10.83	0.62
Methionine	14.97	14.99	1.2	0.99
Valine	150.41	153.41	14.20	0.89
Isoleucine	67.05	70.11	6.78	0.75
Leucine	85.88	90.55	8.84	0.71
Phenylalanine	41.86	44.46	3.07	0.55
Tryptophan	25.27	21.79	2.58	0.34
% of Total AA				
Essential AA	37.99	38.39	1.85	0.88
Nonessential AA	62.02	61.61	1.85	0.88
Glycogenic AA	66.11	65.72	1.65	0.87
Ketogenic AA	10.44	11.02	0.73	0.57
Branched-chain AA	36.08	35.29	1.77	0.75
Essential AA				
Histidine	2.41	2.36	0.14	0.81
Arginine	2.65	2.56	0.29	0.82
Threonine	2.97	3.03	0.20	0.81
Lysine	4.87	5.25	0.48	0.57
Methionine	0.97	0.96	0.04	0.77
Valine	9.81	9.76	0.51	0.94
Isoleucine	4.36	4.46	0.26	0.79
Leucine	5.57	5.77	0.33	0.67
Phenylalanine	2.73	2.85	0.09	0.33
Tryptophan	1.65	1.39	0.12	0.14

[1]Treatment included ad libitum access to corn stalks, followed by daily supplementation of MP at 125% (MP125) or 150% (MP150) of NRC MP requirements for non-pregnant, non-lactating multiparous beef cows.

[2] Greater SEM presented (MP125: n = 6; MP150: n= 5).

Table 4.6. Effects of excess amounts of MP supplementation on circulating AA concentrations

	Treatment[1]			
AA, umol/L	MP125	MP150	SEM[2]	*P*-value
Total AA	1490	1526	84.59	0.76
Essential AA	620.71	636.51	46.09	0.81
Nonessential AA	869.18	898.57	50.45	0.77
Glycogenic AA	946.13	968.27	54.26	0.77
Ketogenic AA	189.72	198.54	17.23	0.71
Branched-chain AA	610.59	668.83	25.81	0.16
Essential AA				
Histidine	49.90	48.79	2.69	0.77
Arginine	48.16	53.58	7.24	0.59
Threonine	36.47	33.37	4.59	0.63
Lysine	58.71	51.05	9.81	0.58
Methionine	17.44	17.07	1.59	0.87
Valine	143.90	147.88	7.72	0.71
Isoleucine	69.75	66.31	6.36	0.70
Leucine	131.01	147.49	10.18	0.26
Phenylalanine	45.80	51.43	2.18	0.09
Tryptophan	19.57	19.53	1.95	0.99
% of Total AA				
Essential AA	41.48	41.73	1.57	0.91
Nonessential AA	58.52	58.27	1.57	0.91
Glycogenic AA	63.69	36.40	1.46	0.89
Ketogenic AA	12.64	12.99	0.69	0.71
Branched-chain AA	41.04	44.05	0.80	0.02
Essential AA				
Histidine	3.36	3.21	0.12	0.40
Arginine	3.21	3.48	0.38	0.61
Threonine	2.42	2.15	0.22	0.40
Lysine	3.84	3.31	0.51	0.47
Methionine	1.16	1.11	0.06	0.53
Valine	9.67	9.76	0.38	0.86
Isoleucine	4.63	4.35	0.26	0.44
Leucine	8.80	9.68	0.52	0.25
Phenylalanine	3.10	3.38	0.13	0.15
Tryptophan	1.29	1.30	0.10	0.98

[1]Treatment included ad libitum access to corn stalks, followed by daily supplementation of MP at 125% (MP125) or 150% (MP150) of NRC MP requirements for non-pregnant, non-lactating multiparous beef cows.

[2] Greater SEM presented (MP125: n = 6; MP150: n= 5).

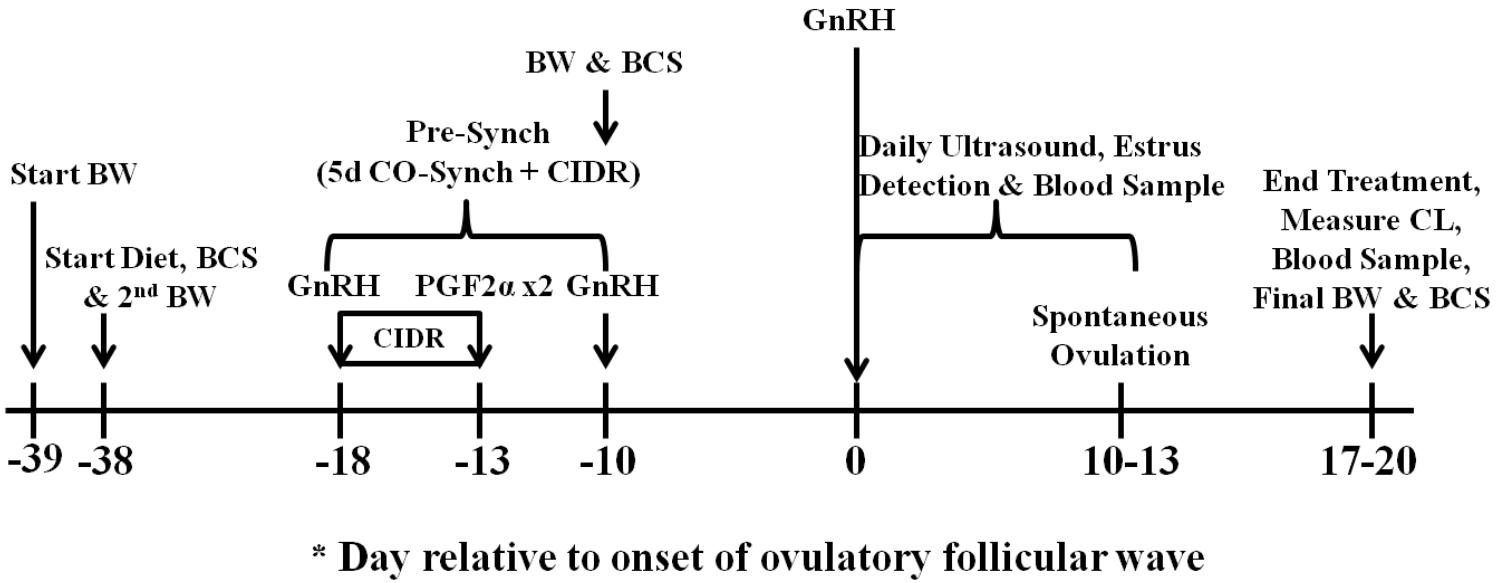

Figure 4.1. Experimental design for dietary treatments (MP125 = 125% MP corn gluten meal, MP150 = 150% corn gluten meal) outlining daily tasks and data collected relative to onset of ovulatory follicular wave on d 0. Protein supplementation and ad libitum corn stalks began on d -38 and were offered until d 37, for a total of 60 d.

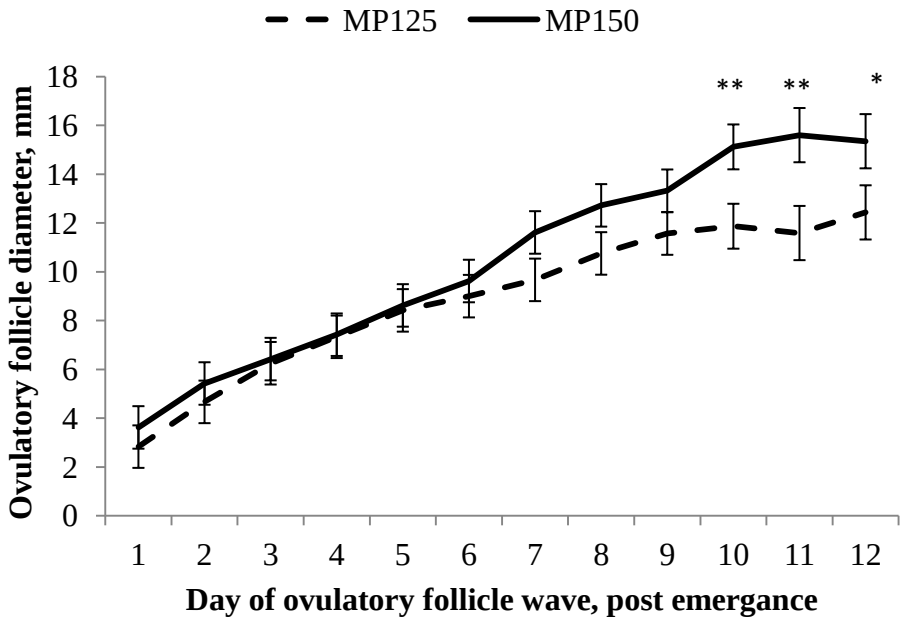

Figure 4.2. Effect of excess amounts of MP supplementation (MP125 = 125% and MP150 = 150% of NRC MP requirements) on ovulatory follicle diameter post-wave emergance. A treatment × day interaction ($P < 0.02$) was observed. P-values for treatment and day were 0.13 and < 0.001, respectively. Days on which ovulatory follicle diameter was significantly different between treatments ($P \leq 0.05$ and $P \leq 0.10$) denoted with ** and *, respectively.

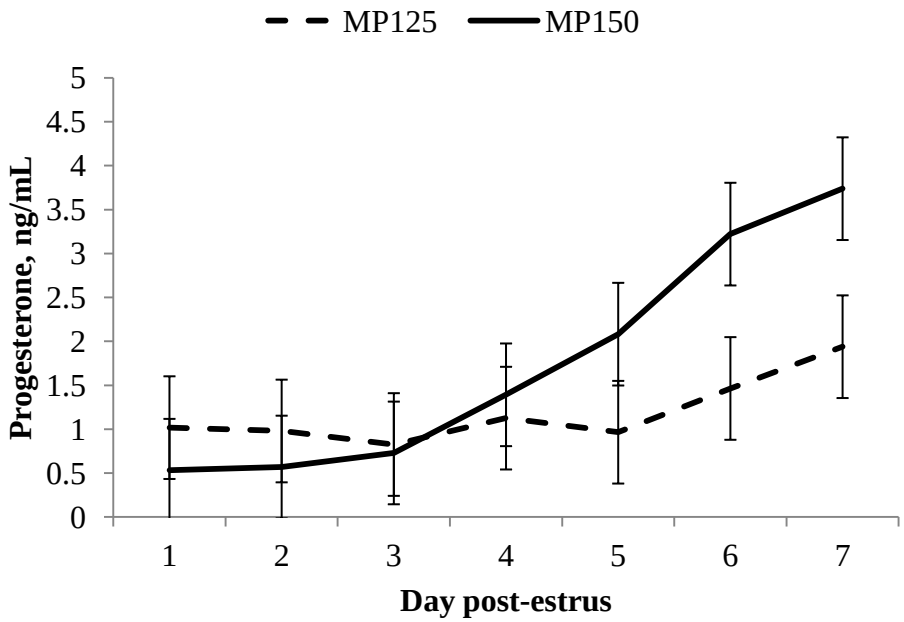

Figure 4.3. Effect of excess amounts of MP supplementation (MP125 = 125% and MP150 = 150% of NRC MP requirements) on circulating progesterone concentrations 7 d post-estrus. An effect of day ($P < 0.001$) was observed. P-values of treatment and the interaction of treatment × day were 0.13 and 0.16, respectively.

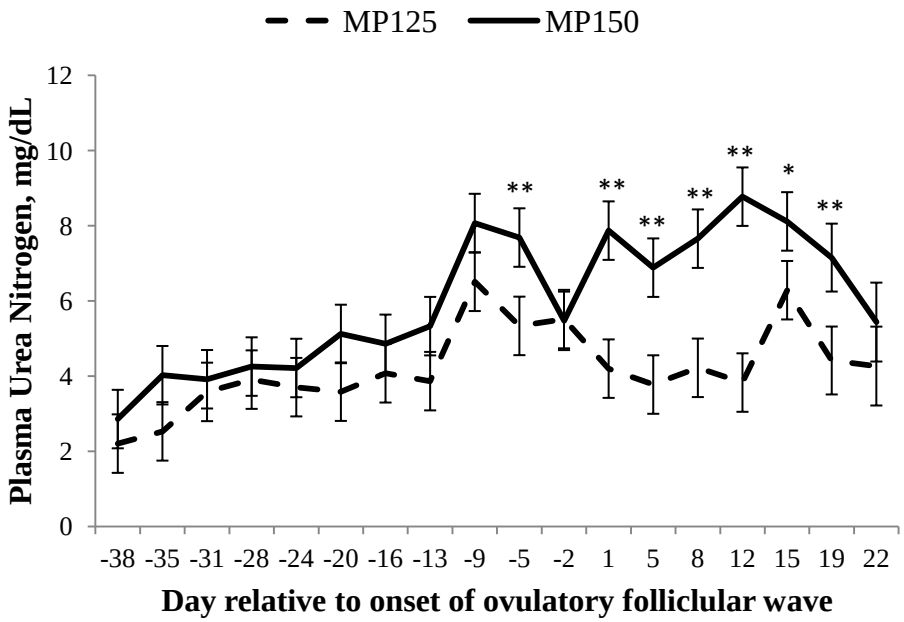

Figure 4.4. Effect of excess amounts of MP supplementation (MP125 = 125% and MP150 = 150% of NRC MP requirements) on circulating plasma urea N (PUN) concentrations. There was a treatment × day interaction ($P < 0.001$) observed. *P*-values for treatment and day were 0.02 and $P < 0.001$, respectively. Days on which PUN was different between treatments ($P \leq 0.05$ and $P < 0.10$) are denoted by ** and *, respectively.

CHAPTER 5.

GENERAL DISCUSSION

Continuous evolution of the ethanol industry has altered production of the traditional corn coproducts producers were once familiar with, into a new generation of coproducts. In an effort to extract further value from the corn kernel, oil extraction during the ethanol production process has resulted in decreased fat and increased CP content of subsequent distillers grains compared to traditional products. Not only do methods of oil extraction alter the nutrient profile of these coproducts but also the feeding value of the products. As when DG were first introduced to the beef industry, producers are questioning how these new low fat coproducts will impact beef cows. Particularly when utilized as an energy supplement for beef cows, producers are likely exceeding protein requirements of the animal; how this excess protein will affect reproduction and fertility is largely unknown. Therefore, the experiments conducted in this thesis were designed to determine the influence of excess protein supplementation on ovarian function and circulating AA of beef cows consuming a base diet of low quality forage.

Even before the increasing availability of high protein ethanol coproducts, protein inclusion was commonly increased in diets of late gestation and early postpartum cows to support nutrient demands of pregnancy and subsequent milk production. Specifically in dairy cattle diets, rumen degradable feedstuffs (such as soybean meal) are commonly the protein supplement of interest being supplied in excess. As inclusion of these moderately degradable protein sources lead to an abundance of ammonia in the rumen, metabolic inefficiencies such as increased concentrations of plasma urea N and lower uterine pH

were observed, which were later associated with suppression of reproductive functions and fertility in high producing dairy cows. However, when diets were composed of coproducts more concentrated in rumen undegradable fractions, fertility was improved. As dairy and beef cow systems have different production and nutrient demands, results are not directly transmittable between these two classes of cattle. Therefore, research was warranted to assess how different types and amounts of fractionated protein supplements affect reproductive and ovarian functions in beef cows.

Due to the differential effects of rumen degradable and undegradable protein on reproductive function in dairy cattle, the first experiment was designed to answer the question of how source of excess protein affects ovarian function of beef cows. Both treatments received excess CP at 150% of MP requirements due to previous research establishing that 150% enhances ovarian function compared to diets feeding supplements to 100% of requirements (Gunn et al., 2014b; 2014c). Furthermore, soybean meal (SBM) was used as the low undegradable supplement to mimic dairy literature. Corn gluten meal (CGM) was utilized as the moderately rumen undegradable supplement as it is a similar (corn) protein that mimics the protein profile and degradability of DG, but without the added fat.

As reported in Chapter 3, feeding excess RUP increased growth of ovulatory follicles post-dominance, and supported larger follicles at luteolysis and at ovulation than SBM-fed cows. However, these larger follicles may be not be superior to the smaller follicles as there was no difference in estradiol concentrations or subsequent CL volumes, and SBM supplemented cows tended to have greater concentrations of progesterone circulating post-estrus than cows offered CGM. The reason for the difference in

progesterone concentrations is not clear, as larger ovulatory follicles have previously been associated with developing subsequently larger CL inducing greater circulating progesterone. Perhaps although the follicles were larger after RUP supplementation, the number of granulosa cells making up these follicles may have been less than the number in the smaller follicles. Since the granulosa cells transform into the large luteal cells in the subsequent CL and produce a majority of progesterone, more granulosa cells may be potentially leading to slightly greater progesterone production.

The second experiment was designed as a follow-up study to Chapter 3 and Gunn et al. (2014c), in order to further elucidate whether the amount of excess RUP supplemented was important to enhancing ovarian functions. Therefore, CGM was supplemented at 125% and 150% of MP requirements to determine if similar results were seen at two excess inclusions. The hypothesis was that RUP offered at 150% would be more beneficial to ovarian function than 125% due to more protein passing to the small intestine, providing more AA for absorption and capable of being used for reproduction. Again, cows fed 150% RUP had larger ovulatory follicles; therefore, the effects of moderately undegradable protein on ovarian function appear to be mediated by amount of RUP potentially due to greater circulation of specific AA.

An interesting observation that occurred in both studies was enhanced ovulatory follicle size and increased circulation of branched-chain AA after supplementation with 150% CGM. Branched-chain AA include leucine, isoleucine and valine, which collectively are the primary AA associated with protein accretion and muscle synthesis. Furthermore, as branched-chain AA (primarily leucine) also influence with ovarian tissues (Yu et al., 2011; Zhang et al., 2011), the potential to increase absorption of these

types of AA may be one way to optimize fertility in beef cows. Still, the ability to connect specific AA to ovarian and reproductive functions warrants further investigation. In monogastric animals, the AA content of the feed consumed closely mimics what AA are absorbed and utilized by the animal. In ruminants the AA profile of protein fed is changed by microbes, and the MCP leaving the rumen does not match the AA profile that was fed (Boisen et al., 2000). However, with the use of high RUP supplements (high in branched-chain AA), the AA absorbed more closely mimics what was fed and may be one way to increase certain AA absorption post-ruminally. Furthermore, with the use of polyunsaturated fatty acids, feed can be encapsulated and protected from degradation in the rumen in order to gain absorbance at the small intestine (Boisen et al., 2000). While these can be more expensive forms of supplementation, if used on specific AA that may be identified to enhance ovarian and reproductive functions, the overall value may outweigh the expenditure.

In dairy cattle, excess CP consumption that leads to PUN greater than 19 mg/dL is a commonly defined as the breakpoint of when suppressed fertility is seen. Collectively the results from Chapters 3 and 4, present data that feeding excess CP from either rumen degradable or undegradable feedstuffs to non-pregnant, non-lactating beef cows does not lead to substantially elevated PUN concentrations, or elicit negative impacts on ovarian function. However, due to the different nutrient demands between non-lactating and lactating beef cattle, these supplements may differentially affect females based on stage of production. Yet, Gunn et al. (2014b) supplemented excess CP from DG to gestating and lactating primiparous heifers and PUN did not exceed the previously defined threshold either. Nevertheless, further research with additional high protein coproducts is

warranted, as is the need to determine if there is a level at which PUN impairs beef cow reproduction as dietary inclusion levels of high protein supplements increase.

As the studies conducted in this thesis only evaluated excess CP effects on ovarian functions, the next step will be to analyze the subsequent effects on fertilization, embryo quality and overall fertility and pregnancy. While this research suggests that supplementing high protein coproducts for 60 d before the anticipated breeding season has favorable results on ovarian function of non-pregnant, non-lactating cows, utilizing these coproducts in lactating beef cow diets may not be economically practical. Ultimately, the energy requirements of lactation can be met with these fractionated coproducts; however, the protein intake and requirements will be substantially greater than in the present studies. In addition, greater inclusions in the diet will lead to potentially unnecessary expenditure depending on current prices. As these low-fat, high-protein coproducts may be practical and beneficial in beef cow maintenance diets, different energy sources may need to be used to develop lactation diets that are more energy efficient and economically feasible.

It has been demonstrated in these and previous experiments that ovarian and reproductive function are influenced by nutritional manipulation. In addition, it appears that specific ovarian mechanisms can be manipulated by designing diets that excel in particular protein constituents. As commodity prices continue to be volatile and producers strive to maintain profitability, several avenues of supplementation may be explored to optimize energy and protein requirements of beef cows depending on stage of production. At this time when used as protein supplements, excess CP supplied from fractionated coproducts does not appear to jeopardize ovarian functions in non-pregnant,

non-lactating beef cows, but the use in lactation diets and the effects on overall fertility need to be further investigated.

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Zhang, Z., Y. Debing, Y. Dingzhong, and W. Zhengchao. 2011. Activation of PI3K/mTOR signaling pathway contributes to induction of vascular endothelial growht factor by hCG in bovine developing luteal cells. Anim. Repro. Sci. 125:42-48. doi:10.1016/j.anireprosci.2011.03.002

Taylor Clair Geppert

Education

Iowa State University
- **Master of Science (**Animal Science)

July 2015
- Specialization: Animal Physiology
- Major Professor: Dr. Patrick Gunn

South Dakota State University
- **Bachelor of Science** (Department of Animal and Range Sciences)

May 2013
- Major: Animal Science (Science Option)
- Minor: Microbiology
- Honors: *Magnum Cum Laude*

Current Research Experience

Animal Physiology *Iowa State University* August 2013–Present

Graduate Research Assistant

Research Proposal:

Over the past decade, the Midwest has been utilizing corn coproducts as a vital part of livestock diets due to the nutrient rich profile of energy and protein. These high quality coproducts can be paired with low quality forages to reduce feed costs for cow-calf and feedlot producers. Research shows that feeding coproducts to beef cows during early lactation improved pregnancy rates. With the emergence of fractionation, coproducts are becoming more concentrated in crude protein (CP) with less fat content. Thus, we need to determine if these low fat, high protein products elicit the same effects on reproduction. Excess crude protein, high in the rumen undegradable fraction, has been found to improve follicular growth. However, the differential impacts of rumen undegradable protein (RUP) and rumen degradable protein (RDP) on beef cow reproduction are unclear. Therefore, the objective of my research was to determine if the amount of CP supplemented, as well as type of CP (RUP or RDP), impacts reproductive parameters of mature beef cows when paired with low quality forage.

Specialized research skills

- Proficient in transrectal ultrasonography for analysis of ovarian function and pregnancy diagnosis
- Artificial insemination, estrous synchronization protocols and hormone administration in cattle
- Developed estrous synchronization programs for research purposes and producers using the Estrus Synchronization Planner from the Beef Reproduction Task Force
- Experience with beef breeding soundness exam collection and analysis
- Experience with embryo transfer protocols and bovine embryo collection
- Jugular and coccygeal venipuncture collection in cattle
- Radioimmunoassay and colormetric assay techniques
- Low-stress cattle handling training and Beef Quality Assurance certified

- Dietary formulation for beef cattle using Beef Ration and Nutrition Decisions Software (BRaNDS)
- Writing SOP and training undergraduate students for on farm and laboratory tasks
- Proficient computer skills such as Microsoft Office, Microsoft Excel, SAS and Camtasia Studio 8 video editing software
- Proficient with design, collection, analysis, interpretation and presentation of research to diverse audiences

Teaching Experiences

Iowa State University

Teaching Assistant – AnS 426: Beef Systems Management Fall 2013–Present
- Provide individual assistance in and out of the classroom with lab assignments
- Assisted with direction of weekly lecture and lab sections
- Responsible for grading lab assignments and recording attendance
- Developed and delivered lecture on estrous synchronization for beef cattle

Teaching Assistant – AnS 411: Issues in Animal Agriculture Fall 2014–Present
- Facilitate senior-level debates about controversial topics in animal industries
- Responsible for evaluating debates and provided constructive feedback
- Attended and evaluated student presentations about current issues

Teaching Assistant – Animal Science 475D: Meat Animal Evaluation Spring 2014
- Assisted with instruction on meat animal evaluation pricing and placing
-Delivered lecture on evaluation techniques for beef, pork and lamb
- Provided instruction and assistance for yield and quality grading classes

Work Experience

Iowa Beef Center Iowa State University January 2014–Present

Extension Graduate Assistant
- Assist with writing of two peer reviewed extension fact sheets on distillers grains as part of a series of six publications
- Assist with setting up meetings, registration, and survey collections
- Attended a series of grazing meetings collecting video footage for cover crop, rotational grazing and grass management educational videos
- Collected video footage of cattle handling demonstrations and low stress cattle-handling for use in creation of educational videos for producers
- Offer assistance to undergraduate employees on video editing and work with extension specialists on production of videos for presentations using Camtasia Studio 8

Reproductive Physiology Research SDSU Fall 2012–Summer 2013

Undergraduate Research Assistant
- Assisted with extension research data collection associated with estrous synchronization protocols, hormone administration, artificial insemination and pregnancy diagnosis
- Assisted with embryo recovery for evaluation of embryonic development and accessory sperm
- Assisted with vaccine trials and liver biopsies for maternal nutrition evaluation

- Offer assistance at artificial insemination training schools by demonstrating the procedure and checking performance of students

Trans Ova Genetics Sioux Center, IA Summer 2012

Summer Production Intern

- Assisted veterinarians with embryo transfer and IVF procedures, including flushing, ovum pick-ups, thawing embryos, transfers and data entry
- Expanded knowledge of skills associated with embryo transfer procedure preparation, palpation, and ultrasound interpretation of donor and recipient animals
- Researched and presented a company update on the physiology of freezing methods, addressing advances and application of current embryo freezing technology

Countryside Vet Clinic, LLC., Kimball, SD Summer 2011

Veterinary Office/ Field Intern

- Worked alongside veterinarian while doctoring large and small animals, preparing administrations and assisting with surgical procedures
- Traveled to on-farm calls and assisted with breeding soundness exams
- Served customers by packaging orders, scheduling appointments and keeping accurate and current inventory

Ruminant Nutrition Research South Dakota State University Fall 2010

Undergraduate Research Assistant

- Assisted with feeding steers, cleaning and maintenance of metabolism crates
- Aided with jugular catheter and rumen cannulation surgeries for blood and rumen sample collection respectively
- Weighed, mixed and delivered daily rations, as well as documented health

Broken Oak Ranch Kimball, SD 2006 – Present

Ranch Associate

- Diversified grain and livestock family farm, assisted in management practices for crops, hay, and production of livestock
- Perform and assist with artificial insemination, synchronization protocols and embryo transfer
- Perform duties associated with herd health, water management, rotational grazing, freeze branding, calving, calf processing and weaning programs
- Clip bulls for local customers and assist with private treaty sale preparation and advertising

Professional Memberships & Certifications

- American Society of Animal Scientists (ASAS) 2013 – 2015
- Associations of Graduate Animal Scientists (AGAS – Iowa State) 2013 – 2015
- Beef Quality Assurance Certified 2014 – Present
- Artificial Insemination in bovine 2010 – Present
- Masters of Beef Advocacy 2009 – Present

Activities and Leadership Experience

ISU AGAS Secretary	2014–2015
Assist with 4-H Youth Beef Roundup	2014
ICA Beef Extravaganza Production Management Judge	2013
SDSU Meat Animal Evaluation Team	2013
SDSU Little International Executive committee and staff	2009–2013
National Cattleman's Beef Association Convention Intern	2012
SDSU Livestock Judging Team	2012
SDSU Intercollegiate Meat Judging Team	2011
SDSU Block and Bridle Club Steer Show Co-Chair	2011
South Dakota National Beef Ambassador	2010
SDSU Sigma Alpha Sorority Co-Rush Chair	2010
SDSU Lead State Academic Leadership Animal Science Representative	2010
South Dakota Junior Angus Association President	2009
SD FFA District and Chapter officer	2006–2009
4-H member in Brule County 4-H	1999–2009

Awards and Scholarships

ISU AGAS Travel Scholarship	2014
John Airy Endowed Graduate Scholarship ISU	2014
SDSU Meat Animal Evaluation Team High Team Individual	2013
Leonard and Violet Wulf Judging Scholarship	2012
SDSU Deans List	2009–2013
SDSU Jackrabbit Guarantee and Opportunity Scholarships	2009–2013
4th High Individual Griswold Cattle Company Collegiate Judging Contest	2011
High Individual Specifications National Western Stock Show, Meats Judging	2011
High Individual Beef Grading Fort Worth Stock Show, Meats Judging Contest	2011
High Individual Specifications Fort Worth Stock Show, Meats Judging Contest	2011
FFA State and American Degrees	2009–2010
SDSU Sigma Alpha Sorority Outstanding Rush Sister	2010
SDSU Little I Champion Beef Showman and Fitting Contestant	2010
National Junior Angus Show Honorable Mention Showmanship Contestant	2009
4-H Key Award Recipient	2009

Abstracts

Geppert, T. C., G. A. Perry, and P. J. Gunn. 2015. Effects of excess dietary MP from corn gluten meal or soybean meal on ovarian function of beef cows consuming low quality forage. J. Anim. Sci. 93(Suppl. 3):313. (Abstr.)

Geppert, T. C., G. A. Perry, and P. J. Gunn. 2015. Effects of supplementing excess amounts of MP from a moderately abundant RUP source on ovarian function of beef cows consuming low quality forage. J. Anim. Sci. 93(Suppl. 3):314. (Abstr.)

Geppert, T.C., A.M. Meyer, and P.J. Gunn. 2015. Effect of excess MP supplementation from corn gluten meal or soybean meal on plasma amino acid concentrations of beef cows consuming low quality forage. J. Anim. Sci. 93(Suppl. 3):W303. (Abstr.)

Geppert, T. C., A. M. Meyer, G. A. Perry, and P. J. Gunn. 2015. Relationship between plasma amino acid profile and ovarian function around the time of ovulation in beef cows. J. Anim. Sci. 93(Suppl. 3):M228. (Abstr.)

Geppert, T.C., A.M. Meyer, and P.J. Gunn. 2015. Effects of increasing supplementation of rumen undegradable protein on plasma essential amino acid concentrations in beef cows consuming low quality forage. J. Anim. Sci. 93(E-Suppl. 2):358. (Abstr.)

Dias, H.P., S.G. Kruse, S.L. Bird, B.J. Funnell, **T.C. Geppert,** E.L. Lundy, P.J. Gunn, and G.A. Bridges. 2014. Incidence of ovulation to GnRH at onset of 5 d CO-Synch + CIDR and impact on reproduction responses. Joint Annual Meeting, ASAS.

Animal Industry Reports

Geppert, T.C., and P.J. Gunn. 2015. "Effect of excess dietary crude protein from corn gluten meal or soybean meal on reproductive function of beef cows consuming low quality forage," *Animal Industry Report*: AS661, ASL R2949.

Geppert, T.C., A.M. Meyer, and P.J. Gunn. 2015. "Effects of increasing supplementation of rumen undegradable protein on plasma essential amino acid concentrations in beef cows consuming low quality forage," *Animal Industry Report*: AS661, ASL R2950.

Manuscripts in preparation

Geppert, T. C., A. M. Meyer, G. A. Perry, and P. J. Gunn. 2015. Effects of excess dietary crude protein from corn gluten meal or soybean meal on ovarian function, and circulating amino acid concentrations of beef cows consuming low quality forage. (In preparation for submission to Journal of Animal Science).

Geppert, T. C., A. M. Meyer, G. A. Perry, and P. J. Gunn. 2015. Effects of supplementing excess amounts of rumen undegradable protein on ovarian function, and circulating amino acid concentrations of beef cows consuming low quality forage. (In preparation for submission to Journal of Animal Science).

Peer Reviewed Extension Publications

Geppert, T.C., and P.J. Gunn. 2014. Ethanol Coproducts for Beef Cattle: Distillers for Beef Cows. Iowa Beef Center. Iowa State University Extension and Outreach fact sheet, IBCR 200D.

Gunn, P.J., **T.C. Geppert**, and D.D. Loy. 2014. Ethanol Coproducts for Beef Cattle: Handling and Storage Considerations. Iowa Beef Center. Iowa State University Extension and Outreach factsheet, IBCR 200E.

Iowa Beef Center – Growing Beef Newsletter

Geppert, T. Effects of excess MP supplementation from either corn gluten meal or soybean meal on ovarian function of beef cows consuming ad libitum corn stalks. April, 2015.

Additional Presentations	***# attended***	
- *Beef It Up Workshop*, 4-H Youth Extension Program	40	2015
- *Carcass Cutout Value*, US Feeds Producer Meeting	40	2014
- *Ultrasound for Reproductive Management*, 4-H Beef Roundup	50	2014